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Challenges and Techniques in Forensic Identification

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You can kiss your family and friends good-bye and put miles between you, but at the same time you carry them with you in your heart, your mind, your stomach, because you do not just live in a world but a world lives in you

-Frederick Buechner-

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Abstract

The identification of human remains is one of the most important topics in forensic medicine. Human identification is a multidisciplinary process which involves mainly forensic pathologists, anthropologists, odontologists, radiologists, and genetics. Various methods are available for human identification, which are based on the comparison of post-mortem data from the corpse and ante-mortem data from the presumed victim.

For many years the techniques of human identification were based only on the comparison of personal belongings or visual identification. This limited the possibility of identification to cases where the cadaver was in good conditions and the possible identity of the deceased was known. With time, identification techniques have advanced. Skeletal and dental remains are still used to create a biological profile in cases where the possible identity is unknown, in order to narrow the number of possible matches available in the future. Further advances include fingerprint analysis, facial reconstruction, and DNA analysis. These techniques increase the possibility of identification in cases of unknown bodies and decomposed, fragmented, or burned human remains.

The advances made in human identification techniques allow the forensic community the possibility to deal with two issues that have great importance: identification of missing persons and disaster victim identification (DVI).

This paper will review the issues of missing persons and mass disasters in forensic science and the various techniques used in the identification process.

1 Introduction

The identification of human remains is one of the most important topics in forensic medicine. Human identification is a multidisciplinary process which involves mainly forensic pathologists, anthropologists, odontologists, radiologists, and genetics. Various methods are available for human identification, which are based on the comparison of post-mortem data from the corpse and ante-mortem data from the presumed victim.

The increasing numbers of missing persons and mass disasters in the last few years brought to attention the importance of forensic human identification. These cases are challenging for the forensics team because often the possible identity of the decedent is unknown. Furthermore, human remains from mass disasters are often fragmented, commingled, and burned, making it hard to determine the number of victims and their possible identities. Identification in both cases is important in order to bring closure to the families, and the forensics team should use all available methods to reach a positive identification.

The process of human identification can be divided into three steps:

- Collecting postmortem (PM) data and establishing the possible identity of the decedent.
- Collecting antemortem (AM) data of the presumed decedent.
- Comparison of PM and AM data.

The first step in the identification process consists in collecting PM data establishing the possible identity of the deceased so that antemortem records

could be obtained for future comparison. Determining the possible identity of the decedent could be very challenging for the forensics team in cases such as disaster victim identification (DVI) and identification of missing persons. In these cases a biological profile of the decedent is created in order to narrow the number of possible matches available in the future. Osteology and odontology are the two main methods used to build the biological profile which includes general information such as sex, age, height, and ethnic origin, as well as individual markers like tattoos and scars. Facial reconstruction is another method that could be used to determine the possible identity of the decedent.

After establishing the possible identity of the decedent and obtaining AM data, three main methods are used to reach definitive positive identification:

- DNA samples extracted from the remains can be compared to DNA samples retrieved from personal belongings or from relatives of the presumed individual. DNA can also be compared to national databases such as CODIS.
- Fingerprints taken from the cadaver can be used for identification only if antemortem fingerprints of the presumed individual are found in national databases like AFIS.
- Postmortem dental records are compared to antemortem dental records of the presumed individual.

The first two chapters of this paper will review the challenges faced by the forensics team in cases of missing persons and mass disasters. Chapters 4-8

will review the methods used for human identification: osteology, odontology, facial reconstruction, fingerprint analysis, and DNA.

2 Missing Persons and Unidentified Bodies

A missing person is a person whose whereabouts are unknown. The phenomenon of missing persons who are not found is constantly growing every year and has become a worldwide problem. Financial problems, abduction, violence, accidents, and suicide are some of the reasons for people going missing[1]. Unidentified bodies represent another large scale problem. The number of cadavers recovered whose identity remains unknown is increasing every year. The growing number of missing persons and unidentified bodies in the last few year could be in part attributed to the growing flow of illegal immigrants who often find their death while trying to reach their destination and are found without any identifying documents[2].

The number of missing persons and unidentified bodies in Italy since 1974 was assessed by a special authority (Commissario Straordinario del Governo per le Persone Scomparse) appointed by the Italian Ministry of the Interior in 2007. Table 2-1 summarizes the total number of people still missing in Italy in the years 1974-2012, 1974-2013, 1974-2014, and the numbers of Italian and foreign citizens missing. The number of people still missing in Italy from January 1st 1974 to December 31st 2014 is 29,234. 20,848 of these missing are foreigners. We can see that the number of missing persons in Italy is increasing every year, and the number of foreign citizens missing in Italy is much higher than the number of Italian citizens missing[3, 4]. Lazio, Sicily, and Lombardia are the regions with the highest number of missing persons. The number of missing persons from 1974-2014 in Tuscany is 1,100[4] (Table 2-2).

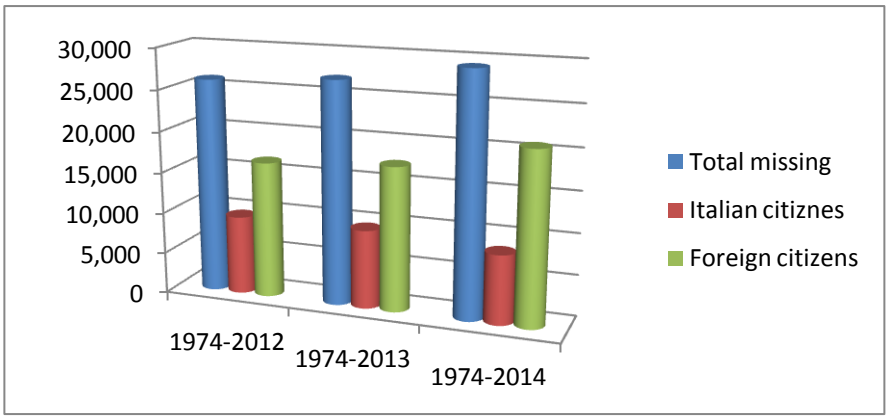

Figure 2.1. Missing persons is Italy in 2012-2014[4]

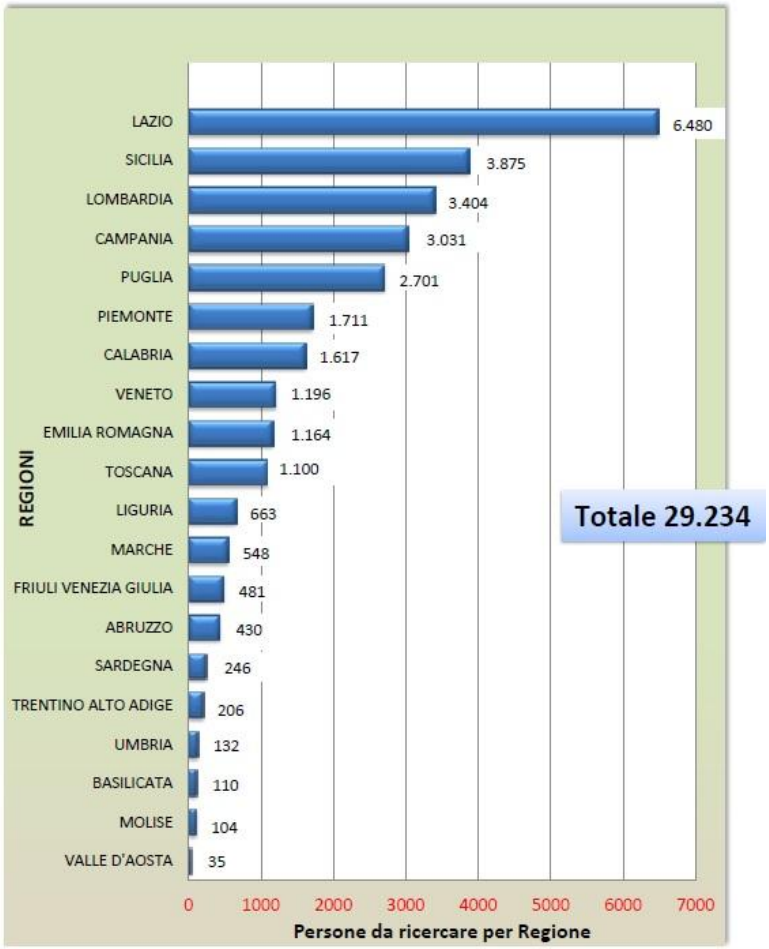

Table 2-1. Missing persons in Italy divided into regions[4].

The number of unidentified bodies in Italy from 1974-2013 was 1,263. In 2014 the number was 1,385. The highest number of unidentified bodies, 681, is found in Sicily, where many unidentified bodies were recovered from the sea after the sinking of a boat of immigrants near Lampedusa[4]. In Tuscany, the number of unidentified bodies from 1974-2014 is 38 (Table 2-3)[4].

Regione	*Recuperati in mare*	*Recuperati in fiume / lago*	*Altro*	*Totale*
ABRUZZO	2		4	6
BASILICATA			2	2
CALABRIA	12	1	10	23
CAMPANIA	5	1	66	72
EMILIA ROMAGNA	2	12	14	28
FRIULI VENEZIA GIULIA		2	8	10
LAZIO	5	45	147	197
LIGURIA	8		20	28
LOMBARDIA		23	79	102
MARCHE	7		10	17
MOLISE	1			1
PIEMONTE		6	24	30
PUGLIA	22	2	27	51
SARDEGNA	10		19	29
SICILIA	629		52	681
TOSCANA	6	8	24	38
TRENTINO ALTO ADIGE		4	13	17
UMBRIA		4	2	6
VALLE D'AOSTA			3	3
VENETO	1	18	25	44
Totale	**710**	**126**	**549**	**1385**

Table 2-2. Unidentified cadavers in the different Italian regions from 1974-2014[4].

A study conducted by Cattaneo et al. at the Institute of Legal Medicine in Milano, LABANOF, examined the extent of the problem of unidentified cadavers and the challenges faced by the forensic team during the identification process in order to try and find a solution[5]. This group studied data from 454 unidentified cadavers who arrived to the morgue between the years 1995-2008. For each cadaver a biological profile was built using anthropological method. Fingerprints were taken from every cadaver and the dental profile was recorded. If a possible match was found, other methods were used to reach a positive identification (Figure 2.1). 62% of the cadavers studied were identified, 47.2% of them were foreign immigrants. 38% remain unidentified. According to Cattaneo et al, the lack of a national database for the comparison between unidentified bodies and missing persons could explain the high number of unidentified bodies. This study also emphasized the important role that forensic anthropologists and odontologists have in the identification process of unidentified bodies.

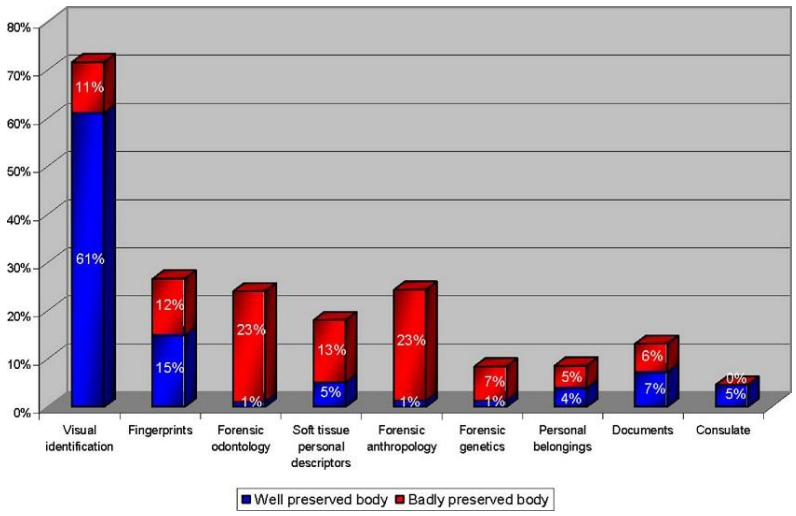

Figure 2.2. Manner of identification of well preserved and badly preserved bodies[5].

In order to try and help with the identification process, a national database for the search of missing persons (Ricerca scomparsi – Ri.Sc.) was launched in 2010[6]. Identifying information of missing persons and unidentified bodies is archived in the database and used for comparison, with the hope of finding a possible match.

The forensics team is responsible of collecting postmortem data from the unidentified body and recording it on a postmortem form which is identical to the antemortem form compiled by the police in missing persons cases (Figure 2.2 and 2.3). Other than a physical description of the body (skin color, hair color, scars and tattoos), the form should include the biological profile of the individual (age, sex, stature, and race), determined by the forensic anthropologist. The dental profile is also recorded. A photo of the decedent should be added when the body is well preserved. In other cases facial reconstruction should be considered.

The information collected by the forensics team is archived in the database (Table 2-4) and compared to the missing person information collected by the police. If a possible match is found, other forensic techniques are used to reach a definitive positive identification.

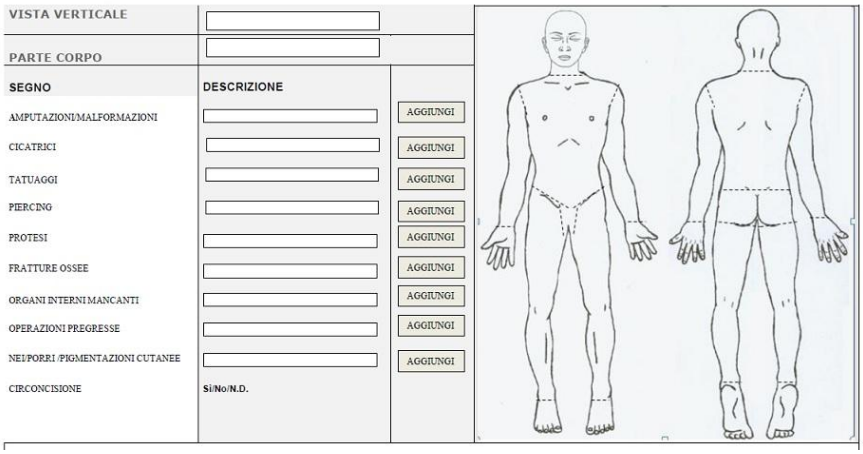

Figure 2.3. An example of an antemortem/postmortem form where particular signs such as scars and tattoos are indicated[7].

Figure 2.4. An example of an antemortem/postmortem form where dental data is recorded[7].

REGIONE	PROVINCIA	COMUNE	LUOGO	SESSO	ETA'	DATA	NOTIZIE
TOSCANA	PISA	PISA	LOC. MONTECALVOLI	F	45-50	09/03/2003	MORTE RISALENTE A 10/30 GG PRIMA PRESENTI INDUMENTI E MONILE - CARNAGIONE CHIARA H 164 CAPELLI ROSSICCI APPENDICECTOMIA, CICATRICE SEDE PUBICA
TOSCANA	FIRENZE	SESTO FIORENTINO	VIA REDI C/O CENTRO COMMERCALE	M	40	18/11/2004	H 1,78, RAZZA CAUCASICA, ITALIANO, CONOSCIUTO COME "ACHILLE IL BARBONE", FORSE NAPOLETANO O SICILIANO, - MORTE PER INTOSSICAZIONE DA MONOSSIDO DI CARBONIO, INDOSSAVA BERRETTO IN LANA MARRONE, GIACCONE NERO, N.2 MAGLIONI BLU, N.1 MAGLIONE NERO, N.1 MAGLIONE MARRONE, T-SHIRT ROSA, JEANS, LACCIO GRIGIO, CALZINI VERDI - TATUAGGIO SU SPALLA SN DI CM.9 RAFFIGURANTE UN PICCOLO FANTASMA SORRIDENTE ARMATO DI FALCE CON ALLE SPALLE UN OMBRA
TOSCANA	FIRENZE	BARBERINO DEL MUGELLO	LOC. VILLANECCHIO A1 KM 251+700	F	20/30	21/06/2006	RESTI
TOSCANA	AREZZO	AREZZO	LOC. INDICATORE	M	60	23/06/2007	H.170, CAPELLI CORTI GRIGI, CARNAGIONE SCURA, NAZIONALITA' VEROSIMILMENTE BULGARA, **SEGNI PARTICOLARI**.: TATUAGGIO SPALLA DX LATERALE, RAFFIGURANTE TESTA DI SERPENTE, TATUAGGIO SPALLA SX LATERALE RAFFIGURANTE DRAGO, CICATRICE ARTO SUP. SX, PARTE INTERNA AVAMBRACCIO.**ABBIGLIAMENTO**: PANTALONI TIPO BERMUDA, SCARPE MOCASSINI.
TOSCANA	PRATO	PRATO	IN CAMPO COLTIVATO LOC. CAPPUCCINI IN VIA DI CAVAGLIANO	F	50/60	13/11/2007	CAUCASICA, CAPELLI CASTANI CHIARI LEGGERMENTE TINTI, ALTEZZA 1,60 CIRCA OCCHI CHIARI, INDOSSAVA UN PAIO DI PANTALONI DI LANA COLORE GRIGIO, UN MAGLIONE DI LANA COLORE NERO, UN PAIO DI SCARPE IN PELLE NERA BASSE, PRIVA DI SEGNI DI RICONOSCIMENTO PARTICOLARI SUL CORPO, PRESUMIBILMENTE DI ORIGINE STRANIERA.

Table 2-3. An example of the information found in the unidentified bodies' registry[8].

3 Mass Disasters

A mass disaster can be defined as any event causing the death or injury of many individuals[9]. Many natural (earthquakes, hurricanes, and tsunamis) and non natural events (terrorism, accidents, and wars) can lead to a mass disaster. Disasters can be considered closed when the number and identities of the victims are known, or open when neither the number nor the identities of the victims are known.

Like other cases of personal identification, the process of disaster victim identification (DVI) requires the comparison of antemortem (AM) and postmortem (PM) records. This process is very challenging for the forensics team for a number of reasons. First, the large number of victims whose identity is not always known, like in the case of open disasters, makes it more difficult to obtain AM records. Second, due to the nature of these events the remains are often fragmented, commingled, and not well preserved, making it harder to determine the number of victims and collect PM data.

Guidelines regarding identification process in mass disasters were developed by the Interpol Standing Committee on DVI[9]. These guidelines describe four stages that should be followed as well was the techniques that should be used for identification (Figure 3.1).

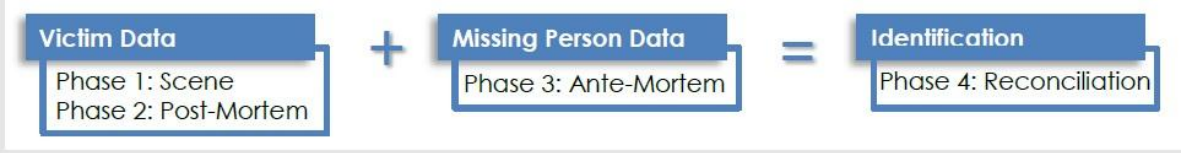

Figure 3.1. DVI process[9].

The four stages of the DVI process are:

- Scene – This step consists in searching for, retrieving and documenting human remains. The remains and other evidence found are photographed and recorded.
- Postmortem – Skeletal and dental remains are examined by the forensic anthropologist and odontologists to try and determine the biological profile of the victim. Fingerprints and DNA samples are taken for future comparison. All the information obtained in the step is recorded on a DVI PM form (Figure 3.2).
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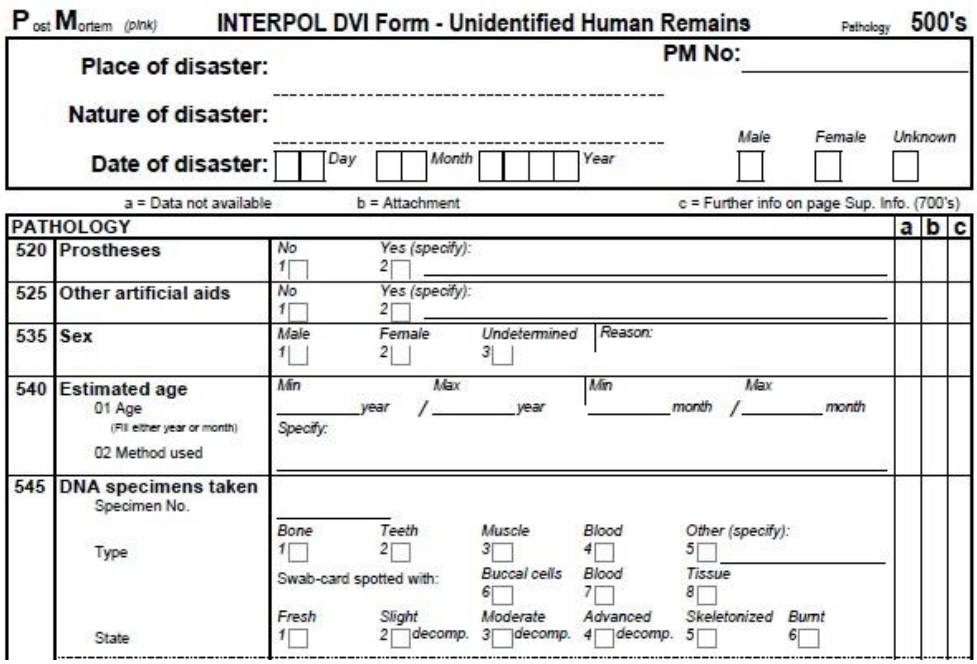


Post **M**ortem (pink) **INTERPOL DVI Form - Unidentified Human Remains** Pathology **500's**

Place of disaster: **PM No:** _____

Nature of disaster:

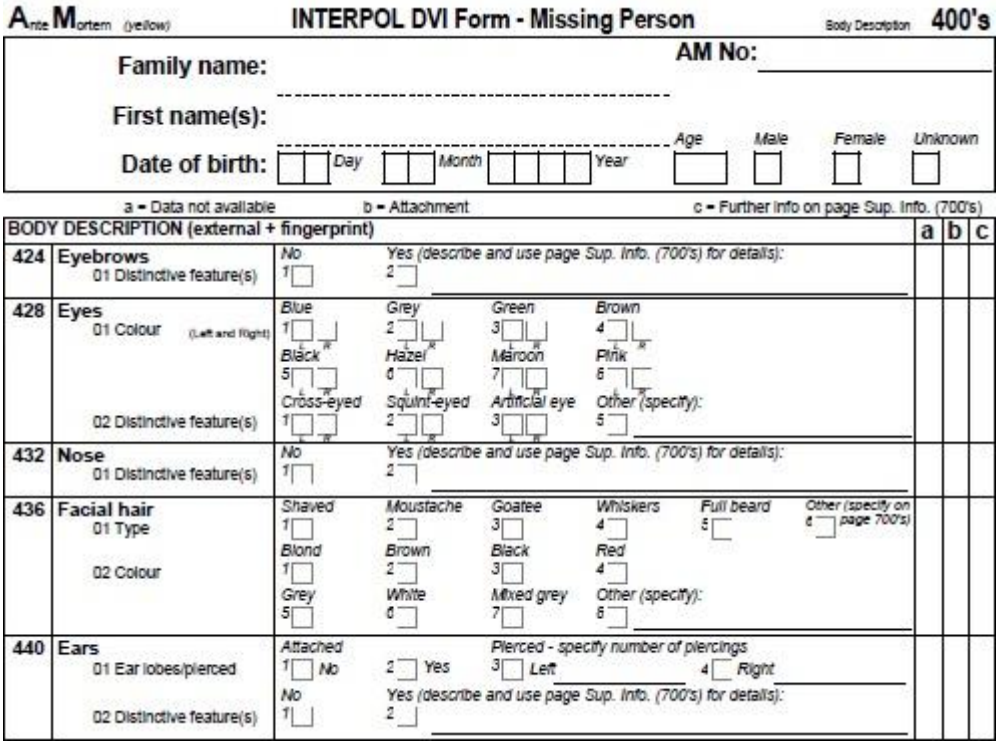
Date of disaster: ☐☐ Day ☐☐ Month ☐☐☐☐ Year — Male ☐ Female ☐ Unknown ☐

a = Data not available b = Attachment c = Further info on page Sup. Info. (700's)

PATHOLOGY			a	b	c
520	**Prostheses**	No 1☐ Yes (specify): 2☐ _____			
525	**Other artificial aids**	No 1☐ Yes (specify): 2☐ _____			
535	**Sex**	Male 1☐ Female 2☐ Undetermined 3☐ Reason:			
540	**Estimated age** 01 Age (Fill either year or month) 02 Method used	Min _____year / Max _____year; Min _____month / Max _____month Specify: _____			
545	**DNA specimens taken** Specimen No. Type State	_____ Bone 1☐ Teeth 2☐ Muscle 3☐ Blood 4☐ Other (specify): 5☐ _____ Swab-card spotted with: Buccal cells 6☐ Blood 7☐ Tissue 8☐ Fresh 1☐ Slight 2☐ decomp. Moderate 3☐ decomp. Advanced 4☐ decomp. Skeletonized 5☐ Burnt 6☐			

Figure 3.2. An example from the DVI PM form[10].

- Antemortem – This step consists in creating a missing person list from family reports or passenger manifests and AM data collection. A detailed description of the presumed victim is recorded. Dental records are collected; DNA samples and fingerprints are taken, as well as any other identifying information. The data collected is recorded on a DVI AM form (Figure 3.3). Collecting AM data could be difficult in open disasters where the number and identities of the victims are not known.
-



Ante Mortem (yellow) **INTERPOL DVI Form - Missing Person** Body Description **400's**

AM No: _____

Family name: _____

First name(s): _____

Date of birth: ☐☐ Day ☐☐ Month ☐☐☐☐ Year ☐ Age ☐ Male ☐ Female ☐ Unknown

a = Data not available b = Attachment c = Further info on page Sup. Info. (700's)

BODY DESCRIPTION (external + fingerprint)			a	b	c
424	**Eyebrows** 01 Distinctive feature(s)	No 1☐ Yes (describe and use page Sup. Info. (700's) for details): 2☐ _____			
428	**Eyes** 01 Colour (Left and Right)	Blue 1☐☐ Grey 2☐☐ Green 3☐☐ Brown 4☐☐ Black 5☐☐ Hazel 6☐☐ Maroon 7☐☐ Pink 8☐☐			
	02 Distinctive feature(s)	Cross-eyed 1☐☐ Squint-eyed 2☐☐ Artificial eye 3☐☐ Other (specify): 5☐ _____			
432	**Nose** 01 Distinctive feature(s)	No 1☐ Yes (describe and use page Sup. Info. (700's) for details): 2☐ _____			
436	**Facial hair** 01 Type	Shaved 1☐ Moustache 2☐ Goatee 3☐ Whiskers 4☐ Full beard 5☐ Other (specify on page 700's) 6☐			
	02 Colour	Blond 1☐ Brown 2☐ Black 3☐ Red 4☐ Grey 5☐ White 6☐ Mixed grey 7☐ Other (specify): 8☐ _____			
440	**Ears** 01 Ear lobes/pierced	Attached 1☐ No 2☐ Yes Pierced - specify number of piercings 3☐ Left _____ 4☐ Right _____			
	02 Distinctive feature(s)	No 1☐ Yes (describe and use page Sup. Info. (700's) for details): 2☐ _____			

Figure 3.3. An example from the DVI AM form[11].

- Reconciliation – Postmortem data is compared to antemortem data in order to reach a positive identification.

The methods used for identification in mass disasters are divided by the Interpol in two categories:

- Primary means of identification include fingerprint analysis, DNA analysis, and comparative dental analysis. These methods are considered to be the most reliable methods for identification.
- Secondary means of identification include personal description, identifying marks like scars and tattoos, personal effects like clothing and jewelry, and visual identification. These are considered to be circumstantial evidence that should be used in support of the primary methods[9, 12].

In 2006, as a response to numerous mass disaster events which required the identification of victims both on a national and on an international level, the Italian police appointed a DVI group which follows the Interpol guidelines. This group is composed of a forensic pathologist, geneticist, odontologist, fingerprint expert, technical personnel, psychologist, and interpreters[13].

4 Forensic Osteology

Forensic osteology is a branch of forensic anthropology that studies human skeletal remains for personal identification.

When skeletal remains are discovered, the forensic osteologist first has to determine if the remains are human, as well as the time period elapsed since death in order to classify the remains as forensic or archeological. An interval of 70 years is used to divide these categories. The forensic osteologist can then begin the identification process by establishing the biological identity of the deceased: sex, age at death, stature, and ethnic origin[14, 15]. It is important to identify which elements of the body are present and their state of preservation as they affect the accuracy with which these four characteristics are determined[16, 17].

Identifying the single bones from the recovered skeletal remains and assigning them to the correct side of the body allows the forensic osteologist not only to reconstruct the body of the deceased, but also to determine the minimum number of individuals present[18]. The type of bone and its position, size, color, and pathological conditions can help separate bones from different individuals. Separating adult bones from juvenile bones can be based on the size of the bones and the state of skeletal development[15]. Separating juvenile bones of different individuals is more difficult and depends mostly on the knowledge and experience that the forensic osteologist has[19]. Finding bones in different stages of development, like a distal ulnar epiphysis and a distinct iliac crest, indicates the presence of at least two juvenile individuals[20]. Table

4-1 summarizes the major centers of ossification and the range of ages in which they appear[19].

Appearance of Secondary Ossification Centres in the Long Bones and Pelvis	
Skeletal Location	**Age Range**
Clavicle	
Shaft only	Birth
Medial epiphysis	12–14 yrs
Lateral epiphysis	19–20 yrs
Humerus	
Shaft only	Birth
Humeral head	2–6 months
Capitulum	By 1st year
Greater tubercle	6 months–2 yrs
Lesser tubercle	4+ yrs
Medial epicondyle	4+ yrs
Trochlea	By 8th year
Lateral epicondyle	10th year
Radius	
Shaft only	Birth
Distal epiphysis	1–2 yrs
Radial head	5th year
Styloid process	By 8th year
Ulna	
Shaft only	Birth
Distal epiphysis	5–7 yrs
Styloid process and olecranon	8–10 yrs
Pelvis	
Ilium, ischium and pubis present	Birth
Femur	
Shaft and distal epiphysis	Birth
Femoral head	By 1st year
Greater trochanter	2–5 yrs
Lesser trochanter	7–12 yrs
Tibia	
Shaft and proximal epiphysis	Birth
Proximal secondary centre	By 6 weeks
Distal secondary centre	3–10 months
Ossification of medial malleolus	3–5 yrs
Distal part of tuberosity starts to ossify	8–13 yrs
Fibula	
Shaft only	Birth
Distal epiphysis	9–22 months
Proximal epiphysis in girls	During the 4th year
Proximal epiphysis in boys	During the 5th year
Ossification of styloid process in girls	During the 8th year
Ossification of styloid process in boys	During the 11th year

Table 4-1. Secondary ossification centers[21].

4.1 Determination of Sex

Male and female skeletons present two main differences. First, male bones are bigger and more robust than female bones. Second, the size and shape of the pelvis is different between the sexes[14, 15, 22].

The skull and the pelvis are the two most sexually dimorphic elements of the skeleton. The most effective sex indicators do not begin to develop until adolescence, making it harder to sex immature remains. Sexual dimorphisms are not always stable throughout life. Walker, for example, showed an association between age and the shape of the greater sciatic notch. At a young age the notch tends to be wider in both males and females, but its shape tend to become more masculine with age, maybe due to vitamin D deficiency[23]. Krogman sexed a sample of 750 adult skeletons. His accuracy rates were 100% when the entire skeleton was present, 95% with pelvis, 92% with skull, 98% with skull and pelvis, 80% with long bones, 98% with long bones and pelvis[24]. According to Mays and Cox, the accuracy of sex prediction is 90% when using only the pelvis and 80% when using only the skull[25].

The female pelvis is wide and shallow with a wide and shallow greater sciatic notch, whereas the male pelvis is high and narrow (Figure 4.1).

Figure 4.1. The wider female greater sciatic notch is on the left, and the narrower and more hooked male morphology is on the right[14].

Other pelvic indicators used to determine sex are reported in Table 4-2. There are various methods for visual evaluation of the pelvis to determine sex. Phenice identified three features that are characteristic to a female pelvis: a subpubic concavity, a ventral arc, and a medial aspect of the ischiopubic ramus[26]. The accuracy of Phenice's method is debatable. Some authors report an accuracy of 59%[27] while others report it is highly accurate[28-30]. Another method used is that of Derrick and Iscán which uses the sacroiliac joint to determine sex[31]. According to their method, which has an accuracy level of 90%, a postauricular sulcus located between the iliac tuberosity and posterior auricular surface is rare in males and very common in females, the postauricular space is narrow in males and large in females, and the iliac tuberosity is absent or pointed in females and mountain shaped in males

(Figure 4.2). Various measurement indices of the pelvis can also be applied to determine sex, although their accuracy is highly variable[21].

Sex Differences in Pelvic Morphology		
Trait	**Male**	**Female**
Pelvis as a whole	Massive, rugged, marked muscle sites	Less massive, gracile, smoother
Symphysis	Higher	Lower
Subpubic angle	V-shaped (<90°)	U-shaped: rounded; broader divergent obtuse angle (>90°)
Subpubic shape	Convex	Concave
Pubic bone shape	Triangular	Rectangular
Ventral arc	Absent, not well defined	Well defined
Obturator foramen	Large, often ovoid	Small, triangular
Acetabulum	Large, tends to be directed laterally	Small, tends to be directed antero-laterally
Greater sciatic notch	Smaller, close, deep	Larger, wider, shallower
Ischiopubic rami	Slightly everted	Strongly everted
Sacroiliac joint	Large	Small, oblique
Auricular surface	Raised	Flat
Postauricular space	Narrow	Wide
Preauricular sulcus	Not frequent	More frequent, better developed
Postauricular sulcus	Not frequent	More frequent, sharper auricular surface edge
Ilium	High, tends to be vertical	Lower, laterally divergent
Iliac tuberosity	Large, not pointed	Small or absent, pointed or varied
Sacrum	Longer, narrower, with more evenly distributed curvature; often 5 or more segments	Shorter, broader, with tendency of marked curvature at S1–2 and S2–5; 5 segments the rule
Pelvic brim, or inlet	Heart-shaped	Circular, elliptical
True pelvis, or cavity	Relatively smaller	Oblique, shallow, spacious

Table 4-2. Sex differences in pelvic morphology[21].

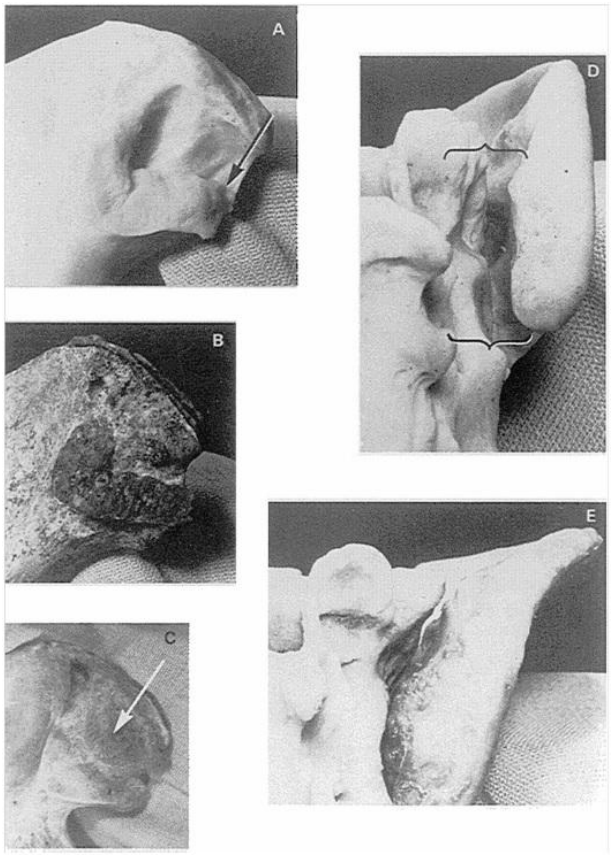


Figure 4.2. Sexual dimorphism in the sacro-iliac between females (A,B and D) and males (C and E). Observe locations of the postauricular sulcus (A), iliac tuberosity (C) and postauricular space (D)[31].

The skull is also used to determine sex. Table 4-3 summarizes the morphological sex traits in the skull. The male skull is usually larger and more robust than the female skull, which is smaller, rounder, and more fragile. Males tend to have more prominent features, like the glabella, supraorbital ridges, and mastoid process. Females present larger frontal and parietal eminences. The nasal bones are larger in males and tend to form a sharper angle in the midline. The nasal aperture in males is higher and narrower with sharp margins. Females have higher and more rounded orbits with sharper

orbital margins. The palate has a parabolic shape in females and U-shape in males. Males present a larger and thicker mandible than females, with greater body weight, and the angle formed between the body and the ramus is less than 125 degrees. Numerous studies have showed low accuracy when using the mandible for sex determination[32-34].

Traits Diagnostic of Sex in the Skull		
Trait	**Male**	**Female**
General size	Large	Small
Architecture	Rugged	Smooth
Supraorbital ridges	Medium to large	Small to medium
Mastoid processes	Medium to large	Small to medium
Occipital area	Muscle lines and protuberance marked	Muscle lines and protuberance not marked
Frontal eminences	Small	Large
Parietal eminences	Small	Large
Orbits	Squared, lower, relatively smaller, with rounded margins	Rounded, higher, relatively larger, with sharp margins
Forehead	Steeper, less rounded	Rounded, full, infantile
Cheek bones	Heavier, more laterally arched	Lighter, more compressed
Mandible	Larger, higher symphysis, broader ascending ramus	Small, with smaller corpal and ramal dimensions
Palate	Larger, broader, tends to U-shape	Small, tends to parabolic
Occipital condyles	Large	Small
Teeth	Large, lower M1 more often 5 cusped	Small, molars often 4 cusped

Table 4-3. Morphological sex traits in the skull[21].

Buikstra and Ubelaker suggested five features that should be used when determining sex from the skull[35]: nucal crest, mastoid process, supraorbital margin, prominence of the glabella, and mental eminence (Figure 4.3). Each

feature is assigned a score from 1 (definitely female) to 5 (definitely male). Walker studied these features on a sample of 304 Europeans and Americans of European and African ancestry and 156 native Americans. He used logistic regression to combine the five features to give formulae that could be used to estimate sex. Using different combinations, Walker was able to correctly classify 77.9%-88.4% of the skulls, with a sex bias of 0.1%[36] (Table 4-4).

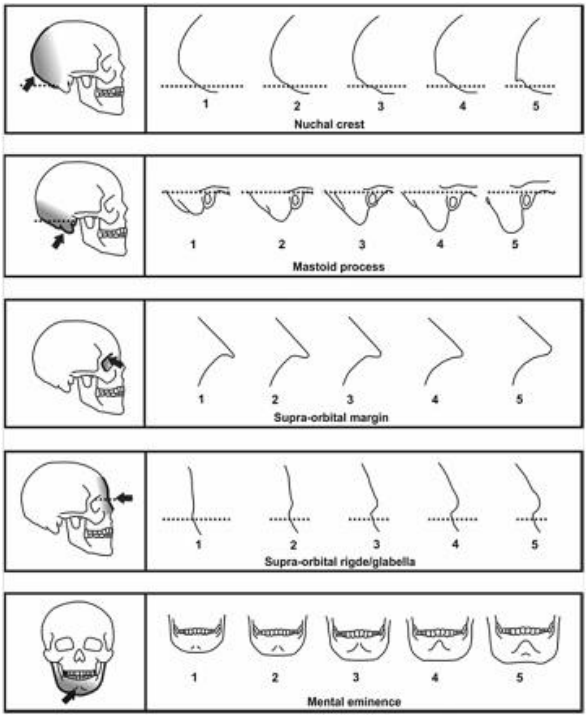


Figure 4.3. Buikstra and Ubelaker's scoring system for sexually dimorphic cranial features[21].

Logistic Discriminant Analysis Equations for Predicting Sex Using Combinations of Cranial Trait Scores for Pooled African American, European American and English Collections (indicated as American/English) and Native American Samples	
Modern Populations	**% Correctly Classified**
Y = 9.128 – 1.375 (glabella) – 1.185(mastoid) – 1.151 (mental)	87.4
Y = 7.434 – 1.568 (glabella) – 1.459 (mastoid)	84.2
Y = 7.372 – 1.525 (glabella) – 1.485 (mental)	84.4
Y = 7.382 – 1.629 (mental) – 1.415 (mastoid)	81.8
Y = 6.018 – 1.007 (orbital margin) – 1.850 (mental)	78.0
Y = 5.329 – 0.7 (nuchal) – 1.559 (mastoid)	79.9
Native Americans	
Y = 3.414 – 0.499 (orbital margin) – 0.606 (mental)	78.0
Y = 4.765 – 0.576 (mental) – 1.136 (mastoid)	73.4
Y = 5.025 – 0.797 (glabella) – 1.085 (mastoid)	76.2

Table 4-4. Discriminant analysis equations for predicting sex[21].

Metric characteristics in the skull have been studied since the 1950s and various formulae have been published[37, 38]. Metric differences between populations pose a problem when using these formulae. Giles and Elliot, for example, used discriminant function methods for determining sex from American whites and blacks, using the Terry collection[38]. Giles also published formulae for determining sex using the mandible[39]. Formulae developed by Spradley and Jantz[40] are now used for the U.S. population, as the individuals from the Terry collection used by Giles and Elliot no longer represent the current living population[21] (Table 4-5). Similar formulae for other populations, like Finns[41] and North Indians[42] have been published.

Discriminant Functions for Estimation of Sex in U.S. Skulls	
Bone	**Classification Function with Stepwise Selected Variables**
American black	
Cranium	(0.71406 x bizygomatic breadth) + (0.43318 x mastoid height) + (–0.59308 x biauricular breadth) + (0.3445 x upper facial height) + (–0.14842 x minimum frontal breadth) + (0.53049 x foramen magnum breadth) + (–0.60805 x orbital height) + (0.32505 x nasal height) – 54.2458 *Accuracy: 90.64%*
Mandible	(0.13874 x bigonial width) + (0.19311 x bicondylar breadth) – 34.6986 *Accuracy: 78.02%*
American white	
Cranium	(0.50255 x bizygomatic breadth) + (–0.07786 x basion-nasion length) + (0.24989 x mastoid height) + (0.19553 x nasal height) + (0.24263 x basion-bregma height) + (–0.15875 x minimum frontal breadth) + (–0.13224 x biauricular breadth) + (0.21776 x glabella-occipital length) + (–0.09443 x frontal chord) + (–0.08327 x parietal chord) + (–0.13411 x occipital chord) – 81.1812 *Accuracy: 90.01%*
Mandible	*(0.15798 x maximum ramus height) + (0.21951 x bigonial width) + (0.06335 x mandibular length) – 35.0107* *Accuracy: 80.80%*

Table 4-5. Spradley and Jantz formulae for estimation of sex in U.S. skulls[21].

Postcranium bones can also be used to determine sex. Spradley and Jantz compared the accuracy of sex estimation using multivariate analysis of the cranium to that of the postcranium. They found that sex can be estimated with an accuracy of 94% when using multivariate analysis of the postcranial bones and only up to 90% when using the cranium. According to these authors, the selection of measurements depends on how much these measurements are expected to differ between males and females and how much correlation exist between them. Width of epiphyses, diameters of head of bones, and

circumferences, for example, are better sex indicators than length or diaphyseal dimension[40].

Morphological and metric methods for estimating sex are less reliable when trying to estimate sex in juveniles because most of the sex differences in the skeleton develop after puberty. Some morphological traits of the pelvis and the cranium could be examined and used when trying to estimate the sex in juveniles. Weaver, for example, found that the auricular surface of the ilium was more elevated in females and non elevated in males. Using this trait, he was able to correctly sex females with a range of 43.5%-75% and males with a range of 73.1%-91.7%[43] (Table 4-6). Numerous researches that were later done using this trait showed accuracies of ~72%[44]. Schutkowski studied four traits of the ilium: angle of the greater sciatic notch arch criterion, depth of the greater sciatic notch, and curvature of the iliac crest (Figure 4.4). He found that 95% of individuals with a narrow greater sciatic notch were boys and 71.4% of individuals with a wider sciatic notch were girls. Using the depth of the sciatic notch he was able to correctly determine the sex of 81.2% boys with deep notch and 76.5% of girls with shallow notch. 73.3% of the boys showed an arch bordering the auricular surface, while an arch crossing the auricular surface was found in 70.6% of the girls. A slight S-shaped curve of the iliac crest was found in 62.1% of the girls, while a pronounced S-shaped curve was found in 81.2% of the boys[45]. Although a number of studies support Schutkowski's findings[46, 47], Vlak et al. found that neither the morphology nor the measurements of the greater sciatic notch are good indicators of sex in juveniles as they were unable to reach accuracies higher than 75% for the sciatic notch traits in a Portuguese sample of individuals under 11 years of age

born between 1805-1972[48]. As for the cranium, some traits of the orbit and the mandible show dimorphism but need further assessment[49].

Auricular Surface Elevation in Fetal and Infant Ilia					
Age Groups	Sex	n	Elevated	Non-Elevated	Percent Correct
Fetal	M	24	2	22	91.7
	F	24	18	6	75.0
Newborn	M	24	7	19	73.1
	F	24	13	11	54.2
Six months	M	32	3	29	90.6
	F	23	10	13	43.5

Table 4-6[21].

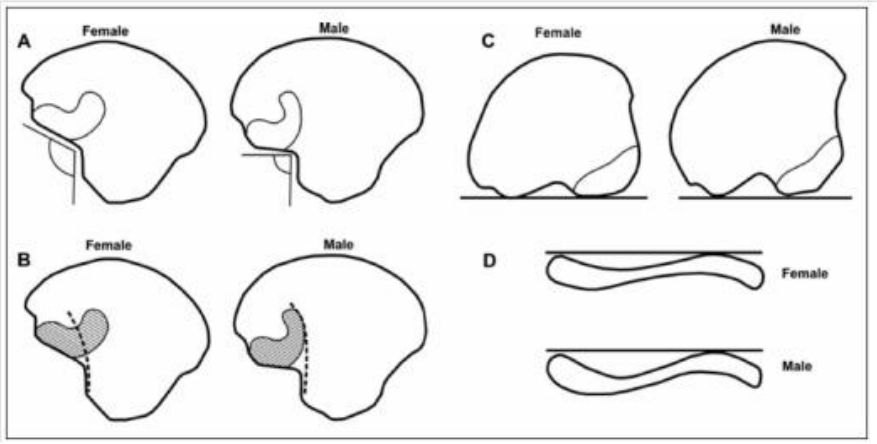


Figure 4.4. Schutkowski's criteria for assessing the ilium in children: (a) the angle of the greater sciatic notch, (b) the arc criteria, (c) depth of the sciatic notch, (d) iliac crest curvature[21].

4.2 Age at Death

There are two types of age that could be determined: chronological age or the number of years lived from birth, and biological age, which is an indicator to the biological maturity of an individual[50]. In juveniles the difference between the chronological and biological age is relatively small. In adults, however, this difference could be significant. Methods for estimating the chronological age, rather than the biological age, are generally applied by forensic osteologists.

Age related changes in the skeleton represent three different stages in life and therefore require different methods for estimating age[21]. The first stage, growth and development, includes children and young adults. Changes in this stage proceed at a predictable rate and pattern and are influenced by genetic and environmental factors. Methods involving the appearance of ossification centers, development and eruption of teeth, and the growth of different parts of the skeleton could be used at this stage[20]. The second stage, equilibrium, follows the cessation of growth. These changes are individual and population specific. Changes in the last stage, senescence and degeneration, are determined by individual factors such as health, occupation, and nutrition[14, 15]. Estimating the age of an individual once growth has stopped is relatively difficult.

Numerous markers from the teeth and skeleton could be used for estimating age in children and young adults. Dental markers include eruption of teeth and their degree of mineralization[51]. Observation of emerged teeth gives the forensic osteologist a rough estimate of age, but a radiographic analysis of the

mineralization of the teeth provides a more accurate estimation[52]. Tooth formation standards, like those of Moorrees[53, 54] and Demirjian[55] are often used to estimate the age of an individual. Another method used is Ubelaker's system which shows the correlation between dental development and age[56] (Figure 4.5). Dental age estimation becomes less accurate at around 14 years of age when the teeth are fully developed. Reppien evaluated the degree of error associated with dental age estimation. He assessed various methods and concluded that the estimated age range can be narrowed to 2-4 years in cases with a developing dentition. The age of a small child could be estimated with a range of 2 years and that of juveniles with a range of 4 years. For adults, Reppien recommended an estimated age range of 10-20 years[57].

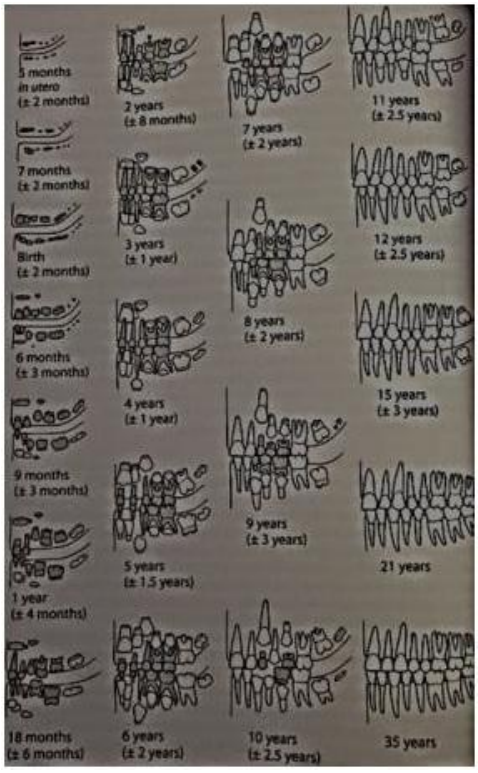


Figure 4.5. Correlation between age and dental development as described by Ubelaker[56]

Skeletal markers include the size and length of the bones and the developmental state of centers of ossification. Estimating the age of an individual using the length of long bones is problematic for a number of reasons. First, with increasing age the accuracy decreases because external factors affect the growth. Second, the tables available for comparison are inadequate as they are based on individuals from at least two generations ago, mostly from white, middle class origins[58, 59]. Primary and secondary centers of ossification are also used for age estimation. Three features of primary and secondary ossification centers can be used: the time at which the center appears, the size and morphology of the center, and the fusion time of primary and secondary centers[15]. These centers develop as spherical or ovoid nodules of bone and can only be identified in their anatomical position, making their use in age estimation limited to cases in which there is sufficient soft tissue to hold them in place[14]. Primary ossification centers of the skull, vertebrae and long bones begin to develop in the embryonic and fetal periods, whereas secondary ossification centers develop throughout postnatal life within cartilaginous templates[20]. The period in which the centers reach their second stage of development and can be recognized by their distinctive morphology varies from bone to bone. For example, most of the bones of the skull, vertebrae, ribs, and long bones can be recognized from mid-fetal life, while carpal bones become recognizable only later in childhood. The accuracy of age estimation increases when using bones in which changes occur in a short period of time.

Figure 4.6. Examples of stages of epiphyseal union, from left to right, open (O), early closure (B), and completely fused (C). In recent union (R), a feint epiphyseal line is present[21].

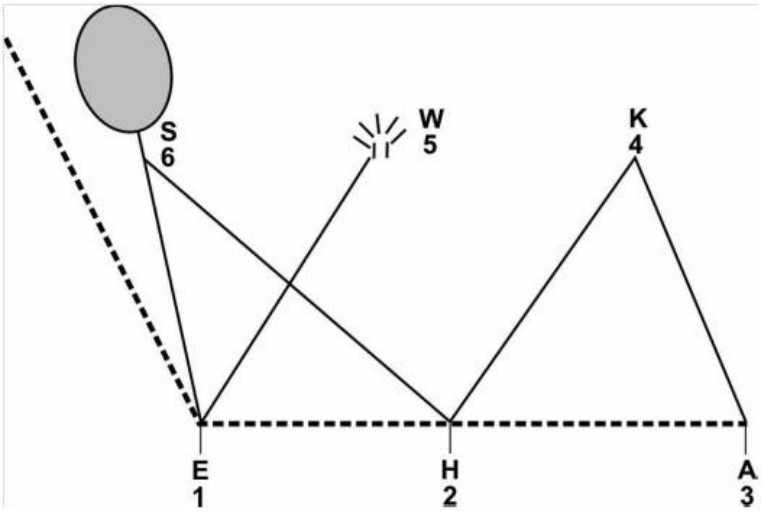


Figure 4.7. Order of epiphyseal closure, starting from the elbow (E), to hip (H), ankle (A), knee (K), wrist (W) and shoulder (S)[21].

Epiphyseal union is more commonly used for age estimation than ossification centers. This process usually begins between 12 to 14 years, earlier in females than in males. In 1924, Stevenson studied epiphyseal union

on a sample of 128 skeletons with an age range from 15 to 28 years[60]. He defined four stages: no union (O), beginning union (B), recent union (R), and complete union (C) (Figure 4.6) and described the sequence of epiphyseal union. The sequence of epiphyseal union was later studied by numerous researchers[61, 62] and is shown in Figure 4.7. The age range of closure for the different epiphysis is shown in Figure 1.8 and Tables 4-7 and 4-8. Differences between populations should be taken into consideration. Crowder and Austin, for example, studied the range of variation in distal epiphyseal union of the tibia and fibula from radiographs from three different North American populations: European-American, African-American, and Mexican-American[63]. No ancestry differences were found in females, who presented complete fusion between the ages of 12-16. Ancestry differences were described, however, in males. African-American and Mexican-American males express complete fusion at 14, while European-American males do not express complete fusion until 16 years.

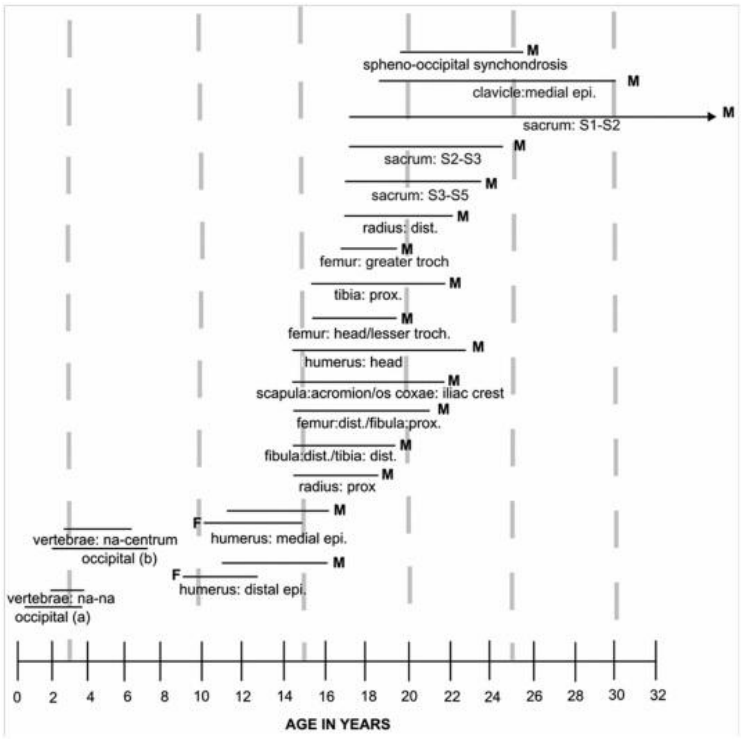


Figure 4.8. Relationship between epiphyseal union and fusion of primary ossification centers and chronological age[21].

Adolescent and Postadolescent Aging According to Epiphyseal Union in Males				
		Open	Partial	Complete
Humerus	Proximal	≤ 20	16–21	≥ 18
	Medial	≤ 18	16–18	≥ 16
	Distal	≤ 15	14–18	≥ 15
Radius	Proximal	≤ 18	14–18	≥ 16
	Distal	≤ 19	16–20	≥ 17
Ulna	Proximal	≤ 16	14–18	≥ 15
	Distal	≤ 20	17–20	≥ 17
Hand	Metacarpals & phalanges	≤ 17	14–18	≥ 15
Femur	Head	≤ 18	16–19	≥ 16
	Greater trochanter	≤ 18	16–19	≥ 16
	Lesser trochanter	≤ 18	16–19	≥ 16
	Distal	≤ 19	16–20	≥ 17
Tibia	Proximal	≤ 18	16–20	≥ 17
	Distal	≤ 18	16–18	≥ 16
Fibula	Proximal	≤ 19	16–20	≥ 17
	Distal	≤ 18	15–20	≥ 17
Foot	Calcaneus	≤ 16	14–20	≥ 16
	Metatarsals & phalanges	≤ 17	14–16	≥ 15
Scapula	Coraco-glenoid	≤ 16	15–18	≥ 16
	Acromion	≤ 20	17–20	≥ 17
	Inferior angle	≤ 21	17–22	≥ 17
	Medial border	≤ 21	18–22	≥ 18
Pelvis	Tri-radiate complex	≤ 16	14–18	≥ 15
	Ant Inf Iliac spine	≤ 18	16–18	≥ 16
	Ischial tuberosity	≤ 18	16–20	≥ 17
	Iliac crest	≤ 20	17–22	≥ 18
Sacrum	Auricular surface	≤ 21	17–21	≥ 18
	S1–S2 bodies	≤ 27	19–30+	≥ 25
	S1–S2 alae	≤ 20	16–27	≥ 19
	S2–S5 bodies	≤ 20	16–28	≥ 20
	S2–S5 alae	≤ 16	16–21	≥ 16
Vertebrae	Annular rings	≤ 21	14–23	≥ 18
Ribs	Heads	≤ 21	17–22	≥ 19
Clavicle	Medial end	≤ 23	17–30	≥ 21
Manubrium	1st costal notch	≤ 23	18–25	≥ 21

Table 4-7. Adolescent and postadolescent aging according to epiphyseal union in males[19, 21].

Adolescent and Postadolescent Aging According to Epiphyseal Union in Females				
		Open	Partial	Complete
Humerus	Proximal	≤ 17	14–19	≥ 18
	Medial	≤ 15	13–15	≥ 16
	Distal	≤ 15	11–15	≥ 15
Radius	Proximal	≤ 15	12–16	≥ 16
	Distal	≤ 18	14–19	≥ 17
Ulna	Proximal	≤ 15	12–15	≥ 15
	Distal	≤ 18	15–19	≥ 17
Hand	Metacarpals & phalanges	≤ 15	11–16	≥ 15
Femur	Head	≤ 15	14–17	≥ 16
	Greater trochanter	≤ 15	14–17	≥ 16
	Lesser trochanter	≤ 15	14–17	≥ 16
	Distal	≤ 16	14–19	≥ 17
Tibia	Proximal	≤ 17	14–18	≥ 17
	Distal	≤ 17	14–17	≥ 16
Fibula	Proximal	≤ 17	14–17	≥ 17
	Distal	≤ 17	14–17	≥ 17
Foot	Calcaneus	≤ 12	10–17	≥ 16
	Metatarsals & phalanges	≤ 13	11–13	≥ 15
Scapula	Coraco-glenoid	≤ 16	14–18	≥ 16
	Acromion	≤ 18	15–17	≥ 17
	Inferior angle	≤ 21	17–22	≥ 17
	Medial border	≤ 21	18–22	≥ 18
Pelvis	Tri-radiate complex	≤ 14	11–16	≥ 14
	Ant Inf Iliac spine	≤ 14	14–18	≥ 16
	Ischial tuberosity	≤ 15	14–19	≥ 17
	Iliac crest	≤ 16	14–21	≥ 18
Sacrum	Auricular surface	≤ 20	15–21	≥ 18
	S1–S2 bodies	≤ 27	14–30+	≥ 25
	S1–S2 alae	≤ 19	11–26	≥ 19
	S2–S5 bodies	≤ 20	12–26	≥ 20
	S2–S5 alae	≤ 14	10–19	≥ 16
Vertebrae	Annular rings	≤ 21	14–23	≥ 18
Ribs	Heads	≤ 21	17–22	≥ 19
Clavicle	Medial end	≤ 23	17–30	≥ 21
Manubrium	1st costal notch	≤ 23	18–25	≥ 21

Table 4-8. Adolescent and postadolescent aging according to epiphyseal union in females[19, 21].

Four areas of the skeleton are normally used to estimate the age in adults: fusion of cranial sutures, the pubic symphysis, auricular surface, and the ribs[14, 51]. The fusion of cranial sutures was widely used in the past to estimate age but the correlation between suture closure and age has been proven to be quite unreliable[64]. Various scoring systems evaluating the degree of suture closure have been developed over the years[50, 65-70], but the method developed by

Acsádi and Nemeskéri is reputed to be the most accurate[70, 71]. According to their method, the cranial sutures are divided into sixteen sections (Figure 4.9) and then scored according to the degree of closure (Figure 4.10). Age is then determined by the mean value (total score based on all the parts divided by 16) (Table 4-9).

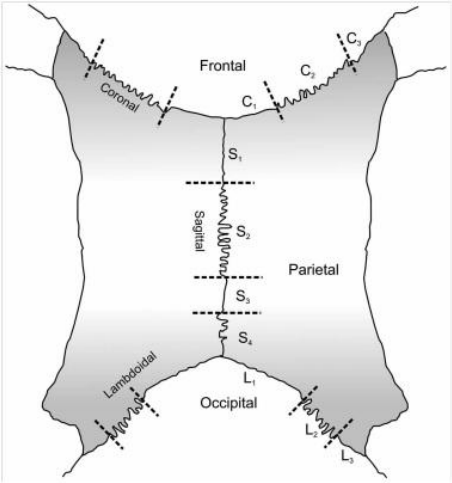


Figure 4.9. The 16 areas of cranial sutures scored by Acsádi and Nemeskéri[21]

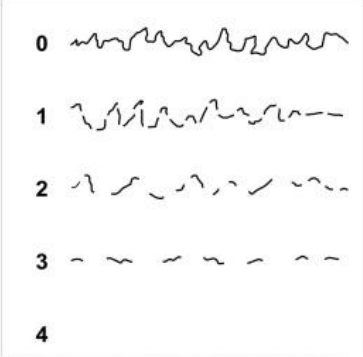


Figure 4.10. Description of the five stages of suture closure. *Stage 0:* Open suture. There is still a little space left between the edges of the adjoining bones; *Stage 1:* Suture is closed, but clearly visible as a continuous, often zigzagging line; *Stage 2:* Suture line becomes thinner, has less zig-zags and may be interrupted by complete closure; *Stage 3:* Only pits indicate where the suture is located; *Stage 4:* Suture completely obliterated, even its location cannot be recognized[21].

Estimation of Age by Sutural Closure				
Mean Closure Stage	Mean Age	SD	Range	Age Category
0.4–1.5	28.6	13.08	15–40	Juvenile–young adult
1.6–2.5	43.7	14.46	30–60	Young–middle adult
2.6–2.9	49.1	16.40	35–65	Young–middle adult
3.0–3.9	60.0	13.23	45–75	Middle–old adult
4.0	65.4	14.05	50–80	Middle–old adult

Table 4-9. Mean values for closure and age according to Acsádi and Nemeskéri[21].

The pubic symphysis is considered the most reliable skeletal indicator of age in adults. Age related changes in the pubic bones were first studied by Todd who described 10 morphological phases and the associated age range[72-75] (Figure 4.11). Both Mckern and Stewart[62] and Gilbert and Mckern[76] presented three component systems based on Todd's studies. Nowadays, these systems are not often used. The most commonly used system today is that of Brooks and Suchey, based on six phases[64, 77] (Table 4-10).

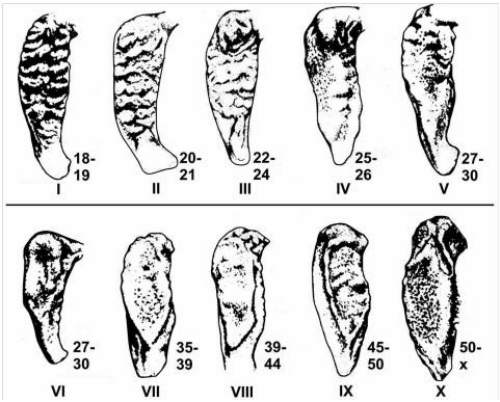


Figure 4.11. Todd's 10 morphological phases with the associated age ranges[62].

Description of the Suchey-Brooks Age Estimation Phases, with Descriptive Statistics for Males and Females
Phase 1
Symphyseal face has billowing surface with ridges and furrows, extends to include pubic tubercle. Horizontal ridges well-marked, ventral bevelling may be commencing. Ossific nodules may occur on upper extremity, but important is that there is no delimitation of either lower or upper extremity *Male: mean = 18.5, SD = 2.1, 95% range = 15–23* *Female: mean = 19.4, SD = 2.6, 95% range = 15–24*
Phase 2
Symphyseal face may still show ridge development. Face has commencing delimitation of upper and/or lower extremities occurring with/without ossific nodules. Ventral rampart may be in early phases as an extension of bony activity at one or both extremities *Male: mean = 23.4, SD = 3.6, 95% range = 19–34* *Female: mean = 25.0, SD = 4.9, 95% range = 19–40*
Phase 3
Symphyseal face shows lower extremity and ventral rampart in process of completion. A continuation of fusing ossific nodules can be present, forming the upper extremity and also along the ventral border. Symphyseal face is smooth or can continue to show distinct ridges. Dorsal plateau complete. No lipping of symphyseal dorsal margin, no bony ligamentous outgrowths *Male: mean = 28.7, SD = 6.5, 95% range = 21–46* *Female: mean = 30.7, SD = 8.1, 95% range = 21–53*
Phase 4
Symphyseal face generally fine grained, but remnants of ridges and furrows may remain. Outline oval is usually complete, but hiatus may occur in upper ventral rim. Pubic tubercle fully separated from the symphyseal face by definition of upper extremity. Symphyseal face may have a distinct rim. Bony ligamentous outgrowths may occur ventrally on inferior portion adjacent to symphyseal face. If lipping occurs it is slight and located on dorsal border *Male: mean = 35.2, SD = 9.4, 95% range = 23–57* *Female: mean = 38.2, SD = 10.9, 95% range = 26-70*
Phase 5
Rim is complete with little or no erosion, some slight depression of the face may be present. Moderate lipping usually found on dorsal border. Prominent ligamentous outgrowths on ventral border. Superior ventral border may show breakdown. *Male: mean = 45.6, SD = 10.4, 95% range = 27–66* *Female: mean = 48.1, SD = 14.6, 95% range = 25–83*
Phase 6
Rim erodes, symphyseal face may show ongoing depression. Marked ventral ligamentous attachments. Pubic turbercle appears as separate bony knob in many individuals. Face may be porous or pitted, with disfigured appearance due to ongoing process of erratic ossification. Crenulations may occur and the shape of the face is often irregular. *Male: mean = 61.2, SD = 12.2, 95% range = 34–86* *Female: mean = 60.0, SD = 12.4, 95% range = 42–87*

Table 4-10. Description of the six phases and age estimation according to Brooks and Suchey's system[21].

The auricular surface is an important indicator of age at death because, as opposed to other skeletal indicators of age, it is more durable and its morphology is not affected by sex or ancestry[78, 79]. Lovejoy et al. described

eight morphological phases with their corresponding age range[80] (Figure 4.12 and 4.13). Based on Lovejoy's work, Buckberry and Chamberlain developed a new scoring method that takes into consideration five characteristics described in Table 4-11[81]. The five characteristics are scored separately and then summed up to create a composite score. These composite scores are grouped into seven different stages (Table 4-12). In 2004, Osborne et al. tested the Lovejoy method and found that the age ranges suggested by Lovejoy et al. were inaccurate[79]. In order to increase accuracy, Osborne et al. modified the age ranges and reduced to six the number of phases.

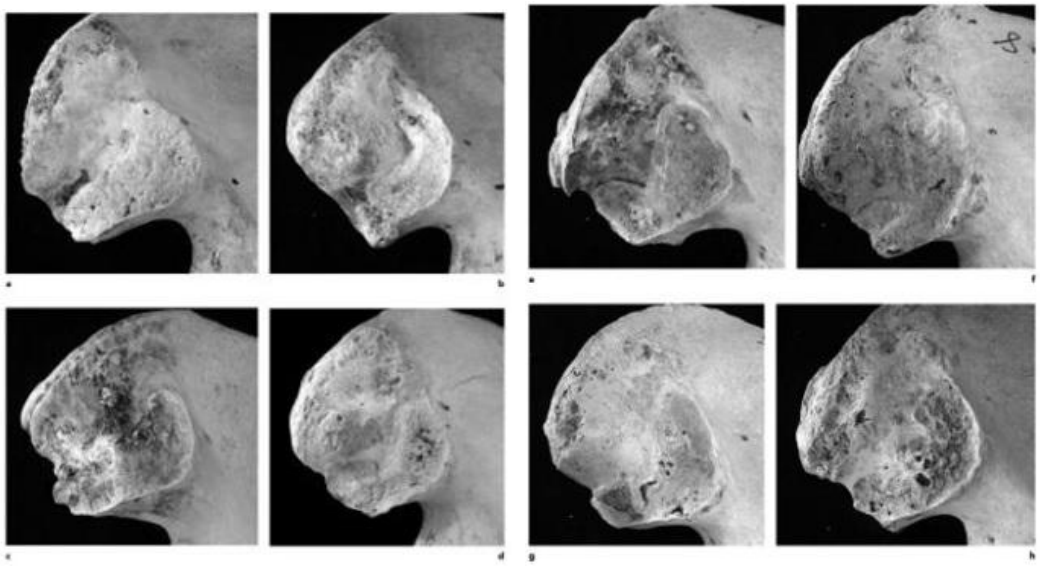


Figure 4.12 and Figure 4.13. The eight morphological phases of the auricular surface as described by Lovejoy et al.[21].

The Revised Auricular Surface Scoring System of Buckberry and Chamberlain (2002)	
Phase	**Description**
Scoring system for transverse organization	
1	90% + of surface is transversely organized
2	50–89% of surface is transversely organized
3	25–49% of surface is transversely organized
4	Less than 25% of surface is transversely organized
5	Transverse organization absent
Scoring system for surface texture	
1	90% + of surface is finely granular
2	50–89% of surface is finely granular; in some areas replaced by coarsely granular bone, no dense bone
3	50% + of surface is coarsely granular, no dense bone
4	Dense bone present on less than 50% of surface, even only one small nodule of dense bone
5	50% + of surface occupied by dense bone
Scoring system for microporosity	
1	No microporosity
2	Microporosity on one demiface only
3	Microporosity on both demifaces
Scoring system for macroporosity	
1	No macroporosity
2	Macroporosity on one demiface only
3	Macroporosity on both demifaces
Scoring system for apical changes	
1	Apex sharp and distinct, auricular surface may be slightly raised
2	Some lipping at apex, but shape of articular margin still distinct and smooth
3	Irregularity occurs in contours of articular surface, shape of apex no longer a smooth arc

Table 4-11. Buckberry and Chamberlain auricular surface scoring system[21].

Composite Score, Stage and Corresponding Ages of the Buckberry and Chamberlain Auricular Surface Method				
Composite Score	**Auricular Surface Stage**	**Mean Age**	**Range**	**SD**
5–6	I	17.3	16–19	1.53
7–8	II	29.3	21–38	6.71
9-10	III	37.9	16–65	13.08
11–12	IV	51.4	29–81	14.47
13–14	V	60.0	29–88	12.95
15–16	VI	66.7	39–91	11.88
17–19	VII	72.3	53–92	12.73

Table 4-12. Stages and corresponding age ranges according to Buckberry and Chamberlain auricular surface scoring system[21].

The sternal end of the ribs is also used to estimate age at death. Iscán developed two methods to determine age using the sternal end of the ribs, a component method and a phase method[82]. The component method was based on morphological changes in three components of the right fourth rib: pit depth, pit shape, and rim wall. The phase method was developed from a sample of 118 white males and consists of eight phases based on age related changes (Figure 4.14). Oettlé and Steyn studied Iscán's phase method on a sample of 339 black individuals from South Africa and found it to be relatively accurate[83]. Changes in the sternal rib ends can also be observed using X-ray and computed tomography images. Dedouit et al., for example, applied Iscán's phase method to two and three dimensional computed tomography images[84].

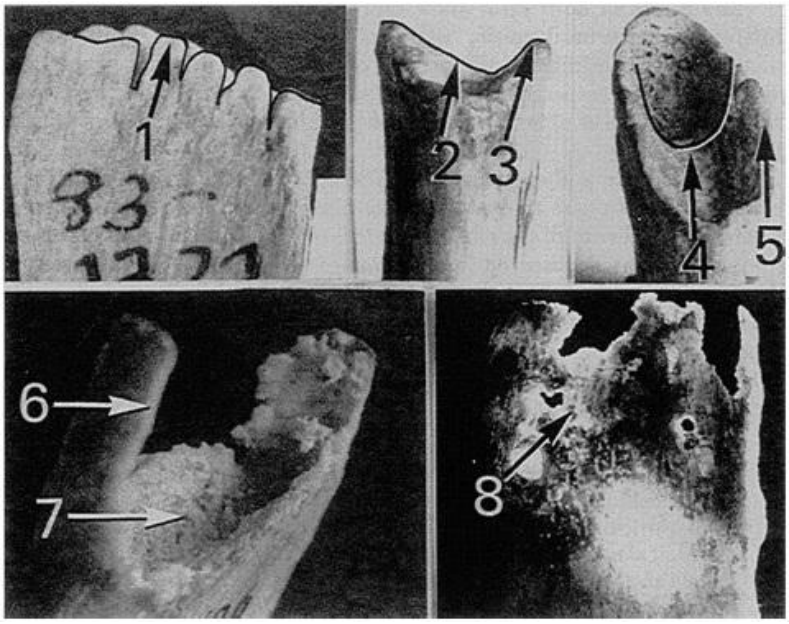


Figure 4.14. Anatomical features used in the estimation of age from the sternal rib end: (1) scallops, (2) V-shaped pit, (3) smooth walls, (4)U-shaped pit, (5) rounded edge, (6) projections, (7) porosity in pit, (8) deteriorated, fragile bone texture[21].

4.3 Stature

Two methods can be used to calculate stature from skeletal remains: Fully's anatomical method[85] and mathematical methods using regression equations. Fully's technique is based on measurements of the basi-bregmatic height, vertebral column length from C2 to S1, physiological length of the femur and tibia, and talo-calcaneal height. Numerous studies have found that the Fully technique underestimates stature[86-88]. To solve the underestimation, Raxter et al. recommended using new soft tissue correction factors with the Fully technique[88]:

$$Living\ stature = 1.009\ X\ Skeletal\ height - (0.0426\ X\ age) + 12.1$$

$$Living\ Stature = 0.996\ X\ Skeletal\ height + 11.7$$

Using these formulae, Raxter et al. were able to correctly estimate the stature of 95% of the individuals in their study with a range of 4.5 cm.

Regression equations are used to establish the relationship between stature and the length of a bone. Formulae that use the lower limb bones and the vertebrae have the lowest error rate[21, 89]. Frequently used regression equations are those published by Trotter and Gleser[90, 91]. In 2010, Wilson et al. published the most recent regression equations for Americans[92] (Table 4-13). Numerous equations for other populations have also been published[93-97].

Equations for Stature Estimation in White and Black Americans			
White Males	**SE**	**White Females**	**SE**
S = 3.574 * Hum + 57.21	5.71	S = 2.534 * Hum + 86.62	5.32
S = 4.525 * Rad + 61.22	5.70	S = 3.530 * Rad + 83.29	4.81
S = 4.534 * Uln + 53.33	5.66	S = 3.346 * Uln + 82.82	4.51
S = 2.701 * Fem + 48.10	5.12	S = 2.624 * Fem + 49.26	3.58
S = 2.891 * Tib + 62.95	5.06	S = 2.351 * Tib + 80.11	4.26
S = 2.832 * Fib + 66.96	5.15	S = 2.487 * Fib + 76.51	4.16
S = 1.728 * (Hum + Fem) + 36.76	5.16	S = 1.656 * (Hum + Fem) + 47.71	3.72
S = 1.525 * (Fem + Tib) + 44.19	4.81	S = 1.330 * (Fem + Tib) + 58.37	4.01
S = 1.556 * (Fem + Fib) + 42.77	4.90	S = 1.382 * (Fem + Fib) + 54.89	3.85
Black Males	**SE**	**Black Females**	**SE**
S = 3.277 * Hum + 65.46	5.72	S = 3.785 * Hum + 47.35	4.56
S = 4.235 * Rad + 63.46	5.07	S = 3.781 * Rad + 75.20	5.01
S = 3.979 * Uln + 62.95	5.79	S = 3.285 * Uln + 80.70	4.18
S = 2.455 * Fem + 56.66	4.84	S = 2.449 * Fem + 54.86	4.34
S = 2.455 * Tib + 75.48	5.03	S = 2.855 * Tib + 58.20	3.83
S = 2.665 * Fib + 69.39	4.53	S = 2.993 * Fib + 55.83	4.29
S = 1.522 * (Hum + Fem) + 50.69	4.83	S = 1.566 * (Hum + Fem + 46.12	4.12
S = 1.295 * (Fem + Tib) + 60.18	4.73	S = 1.340 * (Fem + Tib) + 54.75	3.50
S = 1.341 * (Fem + Fib) + 57.18	4.28	S = 1.365 * (Fem + Fib) + 54.28	3.87

Table 4-13. Regression equations for stature estimation in Americans by Wilson et al.[21].

4.4 Ethnic Origin

Ethnic origin is the most unreliable characteristic of the four characteristics that establish the biological identity of an individual[15]. Metric and non metric traits of the skull, femur, and pelvis are used to determine ethnic origin. In 1990, Rhine published a list of 45 non metric cranial traits for four ancestry groups[98]. A more recent scoring system for eleven non metric cranial traits based on Rhine's work was developed by Hefner and was proved to be useful for determining ethnic origin[99, 100] (Table 4-14). Hefner divided these traits into five categories: assessing bone shape, bony feature morphology, suture shape, presence/absence data, and feature prominence/protrusion[100]. Hefner concluded that a statistical analysis of the distribution and frequency of these traits within and between groups is more accurate than their visual assessment[100].

Anterior nasal spine
Inferior nasal aperture
Interorbital breadth
Malar tubercle
Nasal aperture width
Nasal bone contour
Nasal overgrowth
Postbregmatic depression
Supranasal suture
Transverse palatine suture
Zygomaticomaxillary suture

Table 4-14. Non metric cranial traits used to determine ethnic origin as described by Hefner[101].

Recently, two statistical programs have been developed to determine ethnic origin by analyzing cranial and postcranial measurements[102, 103]. CRANID, developed by Richard Wright, uses 29 measurements and its data bank includes more than 3,000 skulls from around the world[103]. FORDISC, developed by Ousley and Jantz, uses up to 34 cranial measurements and 39 postcranial measurements from numerous data banks[102].

Metric analysis of the pelvis and femur could also be used to estimate ethnic origin[104-107]. Although Iscán reached 88% accuracy with measurements of the bi-iliac breadth, anteroposterior height, and transverse breadth of 400 articulated pelvii, he believed the results might have been influenced by the low socioeconomic status of the individuals used and advised to use them with caution[104]. Patriquin and Steyn studied measurements of a single pelvic bone from a sample of 400 South African blacks and whites, and found that iliac breadth and pubic length were the best indicators of ethnic origin[107]. DiBennardo and Taylor reached 97% accuracy using combined measurements from the pelvis and femur[106].

5 Forensic Odontology

Forensic odontology has been defined by Keiser-Neilson in 1970 as "that branch of forensic medicine which in the interest of justice deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of the dental findings"[108].

The use of dental remains as a means of identification dates back more than 2000 years. It is believed that in 66 A.D., Nero's mistress, Sabina, identified the head presented to her as Nero's wife by the black anterior tooth that she had[109]. Another example is that of Dr. Joseph Warren who was killed in 1775 in the battle of Bunker Hill. His body was identified by his friend and dentist, Paul Revere, by an ivory bridge that he had constructed when Warren was alive[110]. In 1897, an explosion occurred in Bazar de la Charite in Paris, causing the deaths of 126 people. Many of the bodies were identified by dental comparison[111], a process which later led to the writing of the first textbook on forensic odontology, *L'arte dentaire en Medicine Legale*[112].

Forensic odontology is most commonly used in the identification process of deceased individuals, especially in cases where visual identification is not possible due to the conditions of the body; it is one of the primary methods of identification in mass disasters. Dental evidence is useful for a number of reasons:

1. Dental identification can be used in cases of burned, decomposed or dismembered bodies. Tooth enamel is the hardest substance in the

human body; its properties allow teeth to survive extensive trauma, decomposition and direct excessive heat. [113]

2. Dental features such as tooth morphology, tooth position and dental pathology are unique and can be used for comparison. [114]
3. Many individuals have antemortem dental records, such as radiographs and dental charts, from past dental treatments.
4. In the absence of antemortem records, a postmortem dental profile can be made, providing information such as age at death, sex and racial origin.[115]

Comparative dental identification and postmortem dental profiling are the two main methods used in forensic odontology[115, 116].

5.1 Comparative dental identification

The main concept of dental identification is that postmortem dental remains can be compared to antemortem dental records such as radiographs, study casts and written notes indicating details like spaces between teeth, tooth staining or tooth jewelry that may help in the comparison process. Antemortem records can be located when a presumptive identification can be made using a driving license or an ID found on the body; these records have to be of good quality, accurate and comprehensive in order to compare them to postmortem dental remains.

The dental remains are examined by a forensic dentist, who then records the results on a dental chart. Absence of teeth, restorations preformed and the

materials used, fractures, prostheses, and implants should all be documented, as well as other findings. A research performed by Adams showed significant results charting only 28 teeth (third molars were not charted) and only if the tooth is present, missing, or restored. He came to the conclusion that detailed documentation of restorations does not necessarily increase the uniqueness of dental patterns[117]. Postmortem radiographs provide additional non visual details like root canals, cysts, and other pathologies.

The comparison process can begin once the postmortem record is complete. Each tooth needs to be carefully examined as well as the surrounding structures. Various features should be assessed during comparative dental identification (Table 5-1) and similarities and discrepancies should be noted[118]. Discrepancies can be of two types, explainable and unexplainable. Explainable discrepancies like tooth extractions or restorations normally relate to the time elapsed between the antemortem and the postmortem records (Figure 5.1). Unexplainable discrepancies must conclude in an exclusion of identification.

Table 2 — Features examined during the comparative dental identification. This extensive list represents the complexity of these cases, particularly in those instances in which restorative treatment is absent or minimal

Teeth

Teeth present
a. Erupted
b. Unerupted
c. Impacted

Missing teeth
a. Congenitally
b. Lost antemortem
c. Lost postmortem

Tooth type
a. Permanent
b. Deciduous
c. Mixed
d. Retained primary
e. Supernumerary

Tooth position
a. Malposition

Crown morphology
a. Size and shape
b. Enamel thickness
c. Contact points
d. Racial variations

Crown pathology
a. Caries
b. Attrition, abrasion, erosion
c. Atypical variations, enamel pearls, peg laterals etc.
d. Dentigerous cyst

Root morphology
a. Size
b. Shape
c. Number
d. Divergence of roots

Root morphology
a. Dilaceration
b. Root fracture
c. Hypercementosis
d. Root resorption
e. Root hemisections

Pulp chamber/root canal morphology
a. size, shape and number
b. Secondary dentine

Pulp chamber/root canal pathology
a. Pulp stones, dystrophic calcification
c. Root canal therapy
d. Retrofills
e. Apicectomy

Periapical pathology
a. Abscess, granuloma or cysts
b. Cementomas
c. Condensing osteitis

Dental restorations
1. Metallic
a. Non-full coverage
b. Full coverage
2. Non-metallic
a. Non-full coverage
b. Laminates
c. Full coverage
3. Dental implants
4. Bridges
5. Partial and full removable prosthesis

Periodontal tissues

Gingival morphology and pathology
a. Contour, recession, focal/diffuse, enlargements, interproximal craters
b. Colour – inflammatory changes, physiological (racial) or pathological pigmentations
c. Plaque and calculus deposits

Periodontal ligament morphology and pathology
a. Thickness
b. Widening
c. Lateral periodontal cysts and similar

Alveolar process and lamina dura
a. Height, contour, density of crestal bone
b. Thickness of interradicular bone
c. Exostoses, tori
d. Pattern of lamina dura
e. Bone loss (horizontal/vertical)
f. Trabecular bone pattern and bone islands
g. Residual root fragments

Anatomical features

Maxillary sinus
a. Size, shape, cysts
b. Foreign bodies, fistula
c. Relationship to teeth

Anterior nasal spine
a. Incisive canal (size, shape, cyst)
b. Median palatal suture

Mandibular canal
a. Mental foramen
b. Diameter, anomalous
c. Relationship to adjacent structures

Coronoid and condylar processes
a. Size and shape
b. Pathology

Temperomandibular joint
a. Size, shape
b. Hypertrophy/atrophy
c. Ankylosis, fracture
d. Arthritic changes

Other pathologies
a. Developmental cysts
b. Salivary gland pathology
c. Reactive/neoplastic
d. Metabolic bone disease
e. Focal or diffuse radiopacities
f. Evidence of surgery
g. Trauma – wires, surgical pins etc.

Table 5-1. Features examined during comparative dental identification[115].

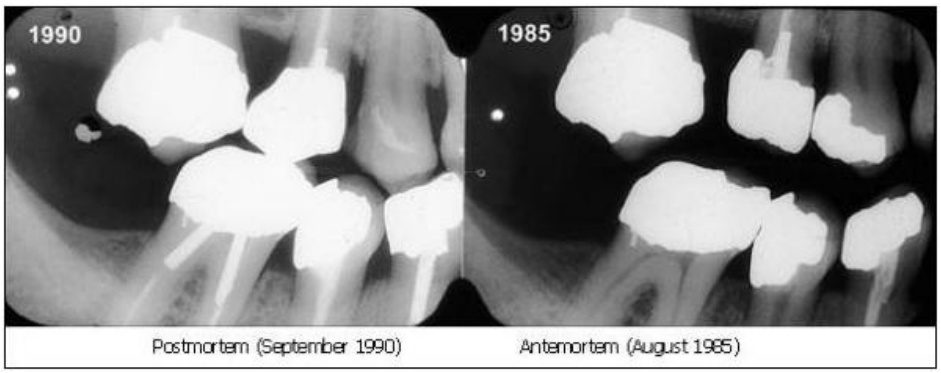


Figure 5.1. The pattern shapes and sizes of individual dental treatments present in one record are compared with similar traits and characteristics in the other record. In this case it was determined that the records originated from the same person. This established a positive identification. Note that there are several discrepancies present, (eg endodontic treatment of teeth LR5 (45) and LR6(46) in the postmortem record) but these can be explained as a result of the time interval between the two records and the additional dental treatments performed during this period[115].

One of the major problems that can arise in the comparison process is the lack of uniformity in charting and tooth numbering systems. There are numerous types of dental charts and tooth numbering systems that provide a diagrammatic representation of the mouth, but the information written in the chart varies from dentist to dentist (Figure 5.2). The three most common numbering systems are the universal system, mostly used in the USA, the ISO system (FDI two digit system), used in most countries, and the Palmer notation method[119] (Figure 5.3). In 1970 Platt and Yewe-Dyer studied the accuracy of dental chartings among dentists in Scotland. They found that 48% of the chartings were correct, 38% were incorrect, and 14% of patient records examined had no charting at all[120]. Using a predetermined charting system can simplify the process.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DENTAL IDENTIFICATION FORM

Figure 5.2. Example of a dental chart[115].

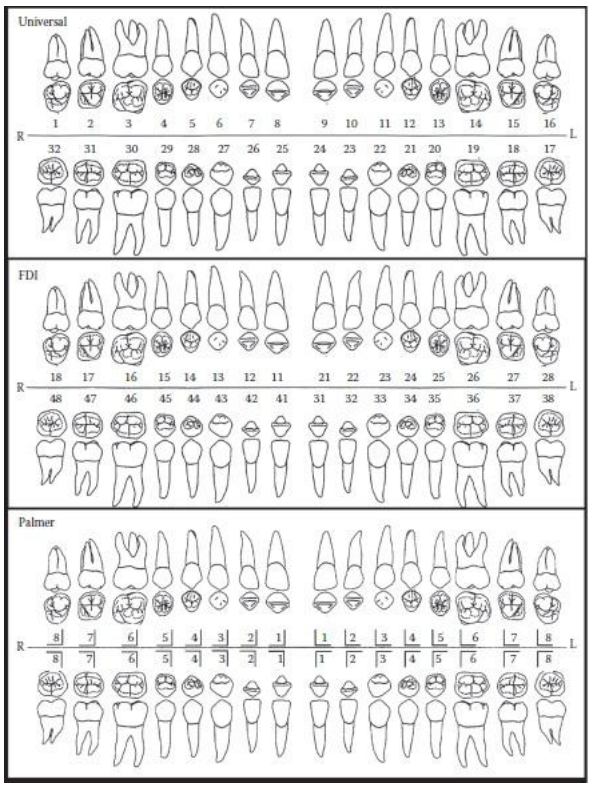


Figure 5.3. The most common charting systems[121].

Dental radiographs play a very important role in the comparison process[122-124]. They are more reliable than dental charts, and show less errors[125]. As opposed to dental charts, which are subjective, dental radiographs are objective records that document the morphology of the dentition and the surrounding bony structures, and they can also provide information about dental treatments like extractions and restorations. In addition, positive identification can be obtained more easily when simultaneously evaluating antemortem and postmortem radiographs than when comparing dental charts. The most common radiographs available as antemortem dental records are bitewing and periapical radiographs, and the orthopantomogram (OPG)[126].

Frontal sinus radiographs can also be used for identification purposes[127, 128]. It is highly recommended that postmortem radiographs would be taken after the antemortem records are made available so the geometry of the antemortem images could be duplicated as closely as possible, facilitating the comparison process[129]. Small changes in horizontal or vertical beam angulation could result in significant radiographic differences, making it more difficult to reach positive identification[130].

Bitewing radiographs can provide details of the crowns of the upper and lower molar and premolar teeth, as well as details of crown morphology, size and shape of pulp chambers, shape of dental restorations, caries, and calculus deposits. Periapical radiographs can show, for example, endodontic treatment, root fractures and implants. These features can be compared with postmortem radiographs that should try to maintain the accuracy of the angulation of the antemortem image. The accuracy of bitewing radiographs in dental identification is very high but in some cases, like identification of children or when there is a long time interval between the antemortem and postmortem radiographs, the accuracy can be lower[123]. OPG was first used in forensic practice for the purpose of identification by Gustafson[131]. It is widely used in dentistry and gives a complete view of the teeth and both jaws in one image[132], but repeating an OPG radiograph postmortem could be very difficult.

Antemortem and postmortem radiographs can be compared or superimposed. Visual comparison consists in matching similar patterns. A number of radiographic patterns have been classified regarding the teeth and the surrounding structures[133, 134]. Two radiographic diagnostic rules have been described by Macdonald in order to facilitate the description of these patterns.

The “5 S rule” includes the analysis of the Shade, Shape, Site, Size, and Surrounding of the area that is being evaluated, while the “3 D rule” includes Diameter, Density, and Displacement[135]. Superimposition consists in digitally overlaying the radiographs, aligning their common features[130] (Figure 5.4).

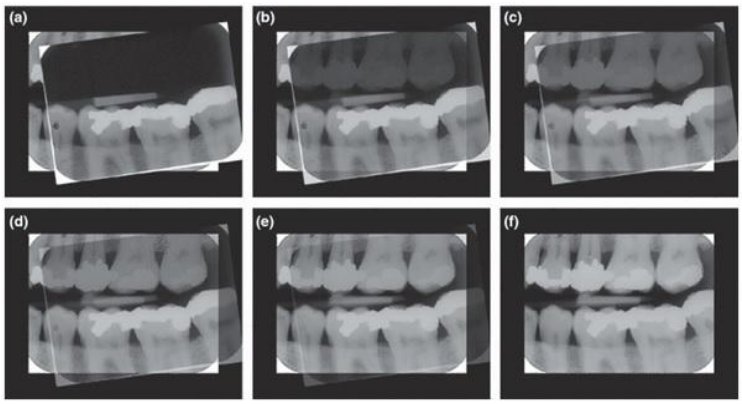


Figure 5.4. At post-mortem examination, an AM radiograph was made available (f). A PM radiograph of the corresponding portion of the mandible was secured, attempting to duplicate the geometry of the AM image as closely as possible. The two radiographs are digitally overlaid and aligned, maintaining the original aspect ratio. Pictures (a)–(f) show how the opacity of the overlying (PM) image is progressively reduced to show the features of the AM image below. This is performed in real-time if required before a tribunal, or included in a Statement of Evidence in the form shown above. (a) PM radiograph opacity 100%; (b) PM radiograph opacity 80%; (c) PM radiograph opacity 60%; (d) PM radiograph opacity 40%; (e) PM radiograph opacity 20%; (f) PM radiograph opacity 0%.[136]

Four conclusions can be made at the end of the comparison process, recommended by The American Board of Forensic Odontology (ABFO)[137]:

- *Positive Identification:* The antemortem and postmortem data match in sufficient detail to establish that they are from the same individual. In addition, there are no irreconcilable discrepancies.

- *Possible Identification:* The antemortem and postmortem data have consistent features, but, due to the quality of either the postmortem remains or the antemortem evidence, it is not possible to positively establish dental identification.
- *Insufficient Evidence:* The available information is insufficient to form the basis for a conclusion.
- *Exclusion:* The antemortem and postmortem data are clearly inconsistent.

Unlike fingerprint identification, where there is a minimum of 12 concordant points in order to reach a positive identification, in dental identification there is no minimum number of concordant points or features required. A single tooth can be used for identification if it contains sufficient unique features[137].

5.2 Postmortem dental profiling

When antemortem dental records are not available for comparison and other methods of identification are not possible, postmortem dental profiling can help narrow down the population pool to which the deceased might belong to, increasing the probability of a potential identification[134]. This profile can provide information on different characteristics of the deceased[138, 139]:

- Age estimation can be achieved using various techniques[140, 141]. The accuracy of any technique used is much better in children than in adults[142]. The analysis of tooth development and comparison with developmental charts is often used to estimate the age of children, with

an accuracy of ± 1.5 years[141, 143]. Third molar development can be used to estimate the age of young adults with an accuracy of ± 4 years, but only between the range of 16-22 years of age[144]. Techniques that study biochemical tooth changes like aspartic acid racemization[145] can be used, but studies have suggested that this technique is highly influenced by heat[146]. Dentin transparency was one of the aspects of age estimation suggested by Gustafson[147] and is considered a very reliable technique[148]. Translucent dentin is the result of increased mineral content in the dentin causing transparency. Beginning in the apex of the tooth, it then spreads coronally throughout life[149]. The length of the dentin affected is measured in mm after extracting the tooth.

- The determination of race includes three populations that have hereditary phenotypic characteristics; Caucasoid, Mongoloid and Negroid (Table 5-2). The presence of Carabelli cusp (Figure 5.5) on the mesolingual side of the first maxillary molar indicates the deceased is most likely Caucasoid. Maxillary midline diastemas and multiple and multicusped premolars are almost always present in Negroids, while Mongoloids have shovel shaped incisors and prominent marginal lingual ridges[121, 150, 151].

Abnormality	Description	Ethnicity?
Incisors:Chisel\Blade	Smooth palatal surfaces with little or no expansion of the proximal margins, small cingulum, rarely a cingulum pit.	Negroid and Caucasoid
Shovel-shaped incisors	Varying degrees of lingual edge thickening giving rise to "shovel" appearance.	Mongoloid
Premolar:Occlusal enamel tubercle/pearl	A nodule of enamel on the occusal surface, often with pulp horn extension; normally lost early in life; can also be present at furcations.	Mongoloid
Maxillari molar Carabelli cusp	Of varying size, this additional cusp can be seen on the mesio-palatal aspect of the first maxillary molar.	Caucasoid
Mandibular molars: Supernumary distolingual root	Third root present on the distal aspect of lower molars.	Mongoloid, Inuit, Native American
Fourth Molars	Additional molar distal to the third molar.	Negroid
Macrodonts	Typically of central incisors.	Caucasoid

Table 5-2. Dental ethnic indicators[150].

Figure 5.5. Cusp of Carabelli on UL first molar[121].

- Gender can be determined by microscopic examination of the pulp tissue for Barr bodies, which are present only in females. This technique is highly accurate and has great value in burnt and mummified remains[152]. Another method that can be used is PCR analysis of DNA from dental pulp followed by the analysis for the amelogenin gene[153].
- Erosion, staining and wear patterns of the teeth might give an indication as to the deceased's occupation and habits (Tables 5-3 and 5-4). Unusual wear patterns are often seen in individuals who, in their line of work, hold items between the teeth to keep their hands free like carpenters, electricians and shoemakers[154], as well as pipe smokers who tend to place the pipe stem in the same location creating a wear pattern in that area over time. Abrasion of the teeth is typical in individuals working in dusty or particulate environments such as flour millers and cement workers[155, 156]. Erosion is the dissolution of enamel or dentine by acidic conditions. Erosion of the labial or bucall surface of the teeth is typical of an extrinsic source of acid and can be found in individuals that work in acidic environments, such as chemical plants and wine tasters[154, 157-161], while intrinsic acid sources, like vomiting, usually cause erosion on the palatal surfaces of the teeth[162]. Staining of the teeth can be seen in individuals working with metals and fertilizers, but also in those who smoke, use tetracycline or chew betel nut[163-165].
- Knowing the health condition of an individual can help determine their identity. A large number of diseases can have an oral presentation, but those affecting the dental hard tissue are the most useful in

postmortem dental profiling (Table 5-5). Gastrointestinal conditions like reflux disease or gastric ulcers, as well as eating disorders with self induced vomiting cause an erosion of the teeth[162, 166-169]. Other conditions, like neonatal jaundice, cause staining of the teeth[170].

- The determination of place of residence and socioeconomic status can be based on the dental techniques, the quality of work and the materials used. Russian dentistry, for example, can be categorized by the use of non precious metals faces with acrylic rather than porcelain crowns, the use of non precious metals in the anterior portion of the dental arch and work of a lower quality than that seen in the West[138].

Occupation	Dental appearance	Cause
Miners Grinders Stone cutters Saw mill workers Flour mill workers	Generalized abrasion	Abrasive dust formation and collection on the occlusal surfaces of the teeth
Sugar refiners Bakers Candy makers[26]	Caries on the labial and buccal surfaces of the teeth	Sugar dust deposits, and stagnates, buccal surfaces of the teeth
Metal workers: *Copper* *Nickel* *Iron* *Tin*	Green staining of dentition Green staining of dentition Fine black lines on teeth Yellow staining of teeth	Inhalation of dust Inhalation of metal fumes leads to deposition of tin sulphide
Chemical workers: *Citric acid, tartaric acid, hydrochloric acid, sulphuric acid, etc*	Smooth polished eroded surfaces	Decalcification of enamel and dentine, due to exposure to fumes. Main effect to labial surfaces. Mastication and tooth brushing lead to loss of tooth substance
Superphosphate industry: *production of phosphorus and hydrogen peroxide*	Fluorosis	Fluorine compounds used in this industry have a direct effect on ameloblasts, specially in younger workers
Battery factory worker	Yellow, gold-brown staining of labial surfaces and erosion of incisors	Cadmium exposure causes the extrinsic staining while the battery acids are responsible for the erosion
Shoe Upholsterers Glass blowers Dress designers Seamstresses Electricians	Abrasion – single of multiple groves found on incisors between their teeth	Holding nails, takes, needles etc, between their teeth
Wine tasters	Erosion, mainly on the labio-cervical surfaces on maxillary incisors and canines	Wine tasting on a daily basis with at least 20 wines tasted per day. Wine pH varies from 3.0 to 3.6 typically

Table 5-3. Depicts the oral manifestations in the oral cavity due to the occupation of the deceased[139].

Habit	Appearance	Cause
Coffee, tea, red wine drinkers	Brown/black staining on labial,lingual and palatal surfaces	Extrinsic staining
Pipe smoking	Unusual patterns of tooth wear in addition to staining	Wear
Painting (canvas)	Unusual patterns of erosion especially on the buccal surfaces	'Gouache' in paint is acidic, and transferred to mouth as brushes are often placed intra-orally
Betel nut use	Staining on buccalsurfaces,usually unilateral	Extrinsic staining
Cocaine	Localised and severe dental caries, particularly in the maxillary premolar region	Testing the purity of cocaine by rubbing it into the gums. Cocaine is often mixed with sugar
Heroin	High caries rate and severe periodontal disease	Oral neglect
Methadone syrup	Rampant caries	Methadone is often delivered as a sugary syrup which adheres to teeth tenaciously

Table 5-4. Depicts the functional and parafunctional habits and their dental effects[139].

Medical condition	Appearance (In dentition)	Cause
Hiatus Hernia[31] Gastric ulcer[32,33] Gastro-oesophageal reflux disease (GORD)	Marked erosion of the palatal surfaces of the maxillary incisors and premolars	Regurgitation or vomiting of gastric contents. Gastric acid has a pH below 1.
Anorexia nervosa Anorexia athletica Bulimia nervosa[34,35] Rumination Chronic alcohol abuse	Marked erosion of the palatal surfaces of the maxillary incisors and premolars	Induced vomiting of stomach contents
Neonatal jaundice	Green to yellowish-brown discolouration of the teeth. Enamel hypoplasia may also occur	Most frequently associated with rhesus incompatibility
Congenital porphyria	Affected teeth show a pinkish-brown discoloration that fluoresces red under UV light	Autosomal recessive inheritance. Circulating porphyrins in the blood are deposited in the dental hard tissues
Congenital syphilis[36,37]	Hutchinson's incisors and Mulberry molars – distinctive shaped teeth	Transmission of *Treponemapallidum* from an infected mother
Drugs used in treatments and dental considerations		
Iron supplements	Black staining of teeth	Surface deposition following oral courses
Minocycline[38-40] Chlorhexidine	Brown/black staining	Precipitation of dietary chromogens

Table 5-5. Depicts the systemic conditions and their associated dental findings[139].

5.3 Other methods of dental identification

Although comparative identification and postmortem dental profiling are the most common methods of dental identification, newer and more innovative techniques can be used. Marking of dentures and dental prostheses can be very useful for identification purposes, although the degree of certainty of identification, in comparison to other methods, is lower due to the fact that dentures are removable[171, 172]. Rugoscopy, the study of palatal rugae, is another technique that can be used. Palatal rugae consist in three to seven ridges radiating out tangentially from the incisive papilla. The pattern of the rugae is unique to an individual and does not change throughout life[173, 174]. A

study conducted by Hemanth showed less than a 1% error rate when using computer software to compare rugae for a human identification[175].

Scanning Electron Microscopy/Energy-Dispersive X-Ray Spectroscopy (SEM/EDS) is a combination of instrumental techniques that provide information about the microstructure and inorganic composition of a sample. These techniques are used for the identification and distinction of tooth structure from other materials, verification of the use of restorative procedures, and identification of inorganic restorative materials[176]. Information provided by these techniques is highly useful when dealing with severely burnt and fragmented remains[177]. An example that shows the importance of these techniques when dealing with incinerated and fragmented remains is that of Calgan air flight 3407. This flight crashed in Buffalo, New York, on February 12, 2009. Many of the victims in the crash were identified through dental records, and the use of microscopy and analytical technology was very helpful in providing identifications that may have otherwise not been possible. A calcined and fragile fragment recovered in the crash area was identified as a lower right mandibular segment containing teeth #30 and 31. Radiographic examination showed traces of possible root canal therapy #30. The clinical crowns had fractured and there was no evidence of presence of restorative materials. Two unidentified victims had a dental profile similar to that of the fragment found and morphological image comparison between the segment and the antemortem radiograph was inconclusive for identification. The use of a stereomicroscope showed that the root of #30 contained small silver blebs, suggestive of root canal sealer. This material was further analyzed using SEM/EDS that showed the presence of elements unique to a

particular brand of root canal sealer, which was documented in the dental records of one of the presumed associated victims, allowing a positive identification[178].

6 Facial Reconstruction

Facial reconstruction, also known as facial approximation, is used in forensic science to help in personal identification when other techniques are unsuccessful[21, 179, 180]. The goal of this technique is to recreate the face of the deceased in the most accurate way to allow recognition and narrow the list of names from which the individual might be identified using other methods[180-182].

Figure 6.1. Facial reconstruction techniques: (A) two-dimensional manual, (B) three-dimensional manual, (C) three-dimensional computerized[183].

The various techniques used for facial reconstruction can be divided into 2D and 3D manual or computerized techniques (Figure 6.1). These techniques are all based on the anatomy of the skull, made up of 22 bones (Figure 6.2), and

the depth of the soft tissue covering it[21, 184]. 3D facial reconstruction techniques can follow one of three approaches.

The first one, developed by Gerasimov in the 1920s, is known as the Russian method[185] and it is based on the anatomy of the face and skull.

The second approach uses only the depth of the soft tissue and is known as the American method[24].

The third approach, known as the Manchester or combined method, uses both the anatomy of the skull and the depth of the soft tissue[181].

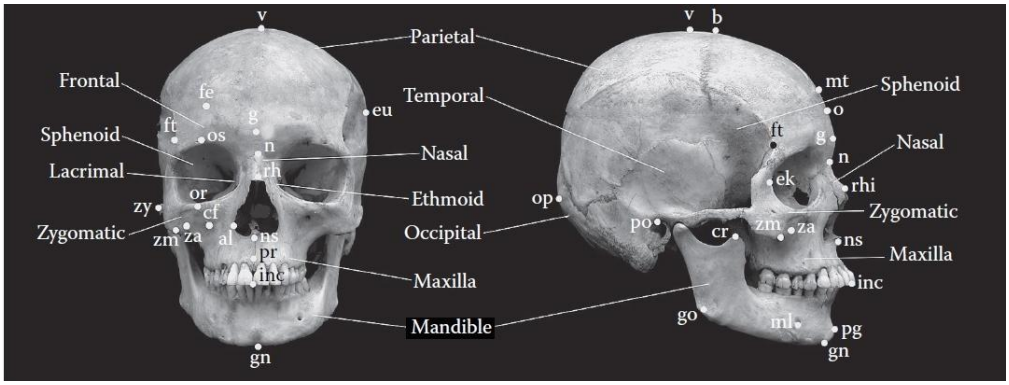


Figure 6.2. Bones of the skull[12].

The accuracy of the various methods has been extensively discussed and studied. Face pools and resemblance ratings are the most used accuracy tests for facial reconstruction[186, 187]. When using the face pool method, volunteers are asked to compare the reconstructed face with a photograph of the target individual and a number of photographs similar to the target individual and choose the face that resembles the reconstruction the most[186, 188]. The

percentage of correct identifications is then compared to what it would be by pure chance. Wilkinson and Whittaker, for example, studied the accuracy rates of the Manchester method using face pool assessment. They analyzed five juvenile female reconstructions and found a recognition rate of 44% where the level by chance was 10%[189]. In another study, Wilkinson et al. studied two adult computerized reconstructions and reported a recognition rate of 71% where the level by chance was 20%[180] (Figure 6.3). Resemblance rating tests require volunteers to score the resemblance between the reconstructed face and the target individual[190]. The resemblance between a reconstruction and the possible targets is then scored taking into consideration elements like age, sex, eye region, nose, and mouth region. This method, however, is considered to be insensitive and misleading[187, 191].

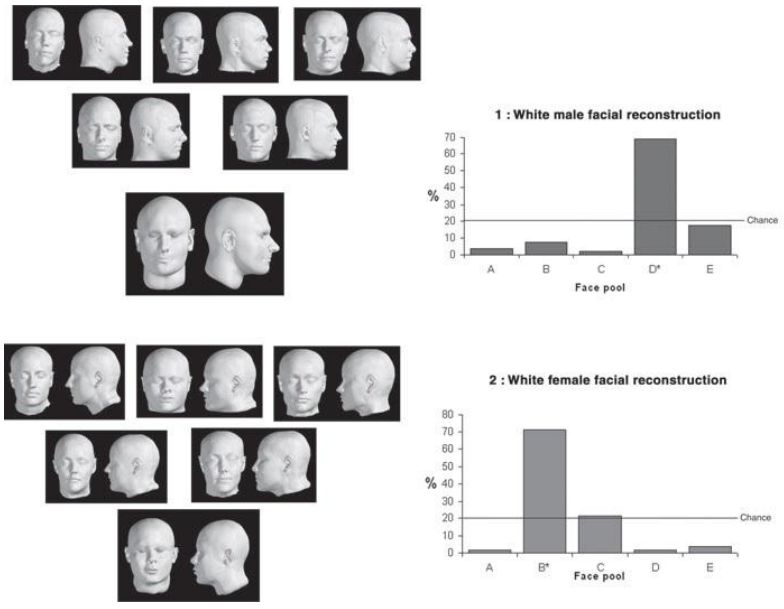


Figure 6.3. Face pool assessments of facial reconstruction accuracy. Top row: reconstruction of a white male skull (1) and a face pool (A–D). Bottom row: reconstruction of a white female skull (2) and a face pool (A–D). Graphs show the results of face pool assessment for 1 and 2 with target faces (*) recording correct recognition rates of > 70% (where the level by chance is 20%)[183].

6.1 Soft Tissue Thickness

Soft tissue thickness (STT) can be measured using various techniques such as radiographs, ultrasound, MRI, and CT. When using radiographs, living individuals can be used to measure STT in the upright position, avoiding the effects of gravity[192, 193]. Ultrasound is believed to be one of the most accurate methods to measure STT[193-195]. MRI[196, 197], CT[198, 199], and cone bean CT images[200, 201] are more recent methods used to measure STT. They allow visualization of soft tissue and bone but they are more expensive. False measurements due to soft tissue swelling for example and imaging artifacts are possible disadvantages of these more recent methods. According to Stephan and Simpson, an MRI scan in the upright position is the most recommended method followed by ultrasound[202].

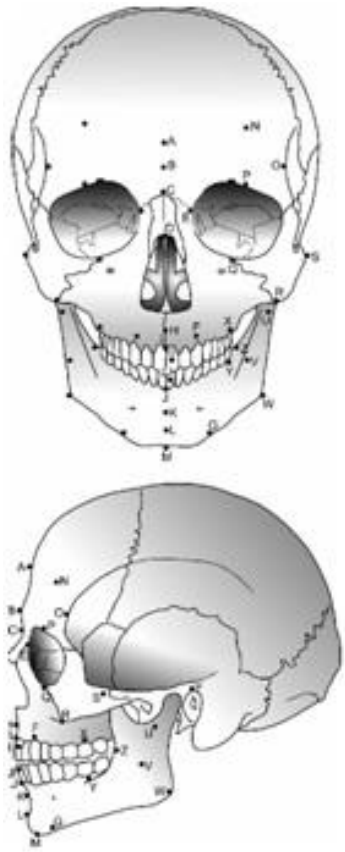


Figure 6.4. Cranial landmarks in anteroposterior and lateral views where soft tissue thickness values are usually recorded[21].

Specific cranial landmarks are used to measure STT values as shown in Figure 6.4. These values vary between different populations and many STT values datasets have been published[186]. These include, for example, North American blacks[203], European whites[195], South African blacks[192, 199], Australians[204], and Egyptians[205]. Stephan and Simpson reviewed published STT values and found no major differences between the sexes and between Caucasoid and non Caucasoid groups. They pooled all the published data and published a single STT table[202, 206] (Table 6-1). Although Stephan and

Simpson did not find major differences between males and females or between races, various other studies demonstrated that differences do exist[186, 196, 199, 203, 207]. Wilkinson concluded that females have more tissue around the cheeks while men have thicker tissues at the brows, mouth and jaw[186].

Generic Soft Tissue Thickness Values		
Landmark	**Weighted Mean**	**Range**
Midline landmarks		
Opisthocranion	6.5	–0.5–13.5
Vertex	5.0	1.5– 8.5
Glabella	5.5	2.5– 8.5
Nasion	6.0	1.0–11.0
Mid-nasal	4.0	0.5– 8.0
Rhinion	3.0	0.0– 5.5
Subnasale	12.5	3.0–22.5
Mid-philtrum	11.0	3.0–18.5
Labrale superius	11.5	3.0–20.0
Labrale inferius	13.0	5.0–21.0
Mentolabial sulcus	11.0	5.5–16.5
Pogonion	11.0	3.5–18.5
Gnathion	8.5	–1.0–18.0
Menton	7.0	0.0–14.0
Paired landmarks		
Mid-supra-orbital	6.0	1.5–10.0
Mid-infra-orbital	7.0	–4.0–18.0
Alare curvature point	9.3	2.5–16.0
Gonion	10.0	–8.0–27.5
Zygion	6.0	3.0– 9.0
Supra canine	9.5	3.5–15.5
Infra canine	10.5	4.5–16.5
Supra M^2	26.0	10.0–42.0
Infra M^2	19.5	6.0–33.0
Mid-ramus	17.5	6.0–28.5
Mid-mandibular border	10.5	–2.5–24.0

Table 6-1. Generic STT values as published by Stephan and Simpson[202].

6.2 Facial Reconstruction Techniques

Before beginning the process of facial reconstruction, the forensic anthropologist needs to collect all the possible information from the skull, like

the sex and age of the individual, as well as determine the facial morphology[24, 186, 208]. The forensic anthropologist should look for asymmetries, unusual landmarks, or bony pathologies that could affect the appearance of the individual's face. Since the reconstruction is not normally done on the original skull, the next step is to create an exact replica of the skull. Plastic or acrylic casts are usually used[181, 186] (Figure 6.5). Three dimensional models produced from CT data, surface scans, or stereolithography[209-211] are used when a computer-based facial reconstruction is planned or when there is no access to the original skull (Figure 6.6).

Figure 6.5. Plaster and acrylic skull replicas[212].

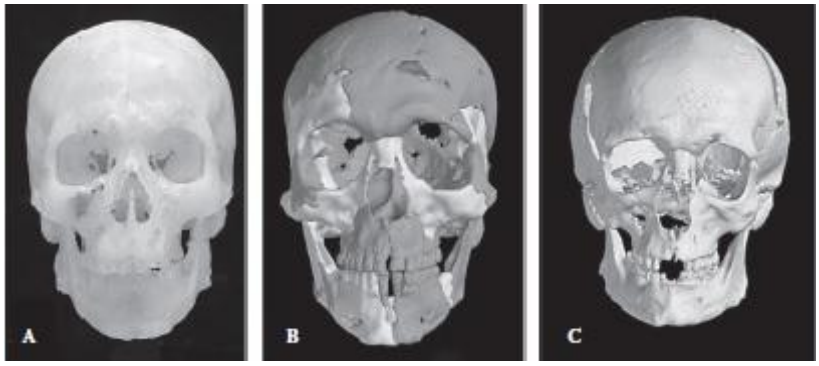


Figure 6.6. Replica skull (A) produced from (B) reassembled CT data or (C) laser scans[212].

Two dimensional facial reconstructions can be manual or computerized. Manual 2D reconstructions consist in drawing the face on an image of the skull[24, 213] (Figure 6.7). Numerous computer programs are available for computerized 2D facial reconstructions. These programs allow adding sketched or photographic features to an image of the skull[214-216] (Figure 6.8).

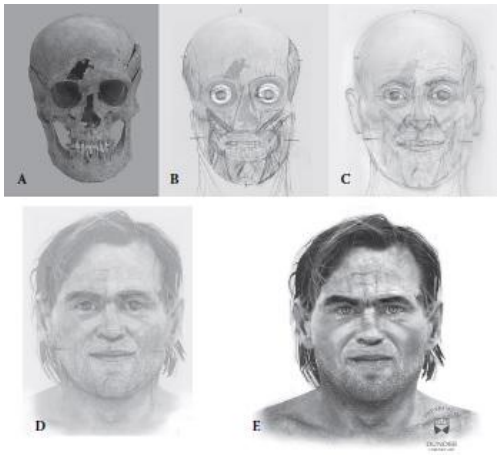


Figure 6.7. Manual two-dimensional facial reconstruction. Tissue depth measurements and anatomical structures (B) are drawn over a photograph of the skull (A). The facial features are then drawn over the muscle structure (C), and these facial proportions and morphology used as a template (D) to create a realistic face (E)[212].

Figure 6.8. Computerized two-dimensional facial reconstruction. A = photograph of the unidentified skull, B = photograph of the skull with superimposed facial reconstruction. C = facial reconstruction produced from a photographic database of facial features[212].

The most popular approach today for 3D facial reconstruction techniques is the combined approach which takes into consideration both the anatomy of the skull and STT. When performing a manual 3D facial reconstruction, pegs indicating soft tissue depths are positioned at anatomical landmarks (Figures 6.4 and 6.9), facial muscles are modeled (Figure 6.10), and specific facial features are determined based on the anatomical details of the skull[21, 183]:

- Eye - The morphology of the eye depends on the position of the medial and lateral canthi and on the position of the eyeball in the orbit[217-219].
- Nose - The width of the bony nasal aperture is about 60% of the total nose width[220, 221]. Gerasimov suggested a two-tangent method to predict where the nose tip should be[220, 222]. The shape of the nose tip reflects the shape of the superior portion of the nasal aperture[223].
- Mouth - The shape of the mouth is related to the dental pattern and the facial profile[220]. Prominent teeth, teeth height, and prognathism are

correlated with thicker lips[186, 220, 224]. The width of the mouth is believed to be equal to the interlimbus distance[224] and the distance between the canines is about 75% of the width of the mouth[225]. The mouth corners are situated below the infraorbital foramina[226] and the oral fissure is located at the lower quarter of the maxillary central incisors[227].

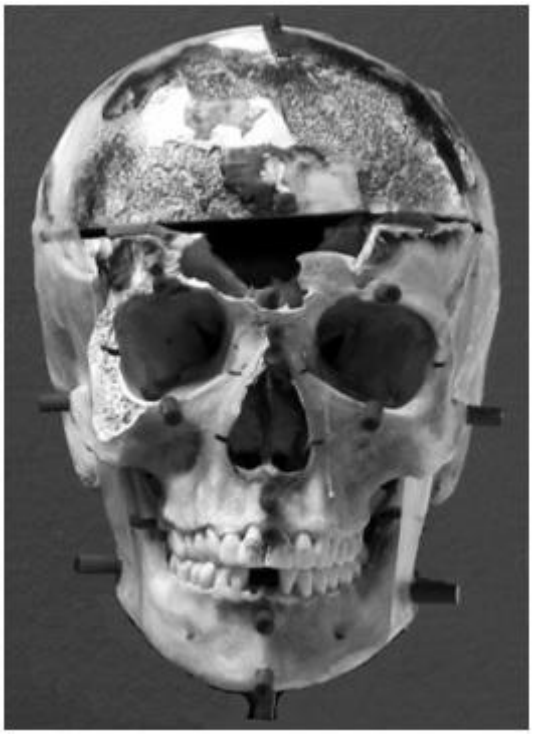


Figure 6.9. Pegs positioned at various craniofacial landmarks, to indicate soft tissue depth[21].

Figure 6.10. Building up of individual facial muscles[21].

Adding the skin layer over the muscles is the last step of the process (Figure 6.11).

Figure 6.11. Composite image showing the skin layer placement in relation to the sculpted musculature and tissue depth pegs for facial reconstruction[183].

Various computerized 3D facial reconstruction methods have been developed over the years[184, 228]. Like all other facial reconstruction techniques, computerized 3D techniques are also based on the connection between the anatomy of the skull and STT. These techniques follow the same chronological steps[184] (Figures 6.12 and 6.13):

- Anthropological examination of the skull in order to determine the age, sex, and race of the individual[186, 208, 229].
- Creating a digitized copy of the skull using laser scanning systems or CT systems[230-232].
- Determining the craniofacial model - Each technique has its own specific craniofacial model (CFM) that codes for different facial shapes. Every CFM has three components: a craniofacial template containing the facial knowledge, craniofacial information containing the anatomical knowledge, and craniofacial deformation containing the sculpting knowledge[184].
- The target skull representation is related to the CFM chosen. Some methods, for example, use virtual pegs to indicate STT[232] while other methods use a simulated tissue growth algorithm[230, 233, 234].
- Skull registration is the next step. This step consists in combining the CFM with the target skull, resulting in the reconstruction of the face[184].
- The last step, texturing, helps generate a more life-like appearance by adding skin color or eye color for example[232, 235].

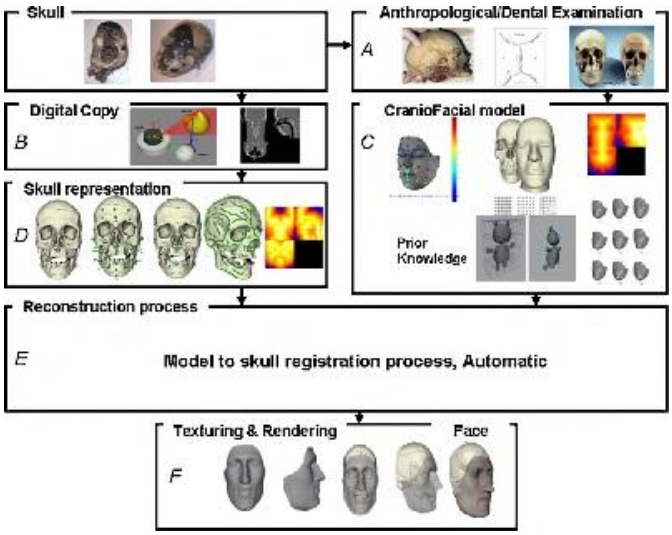


Figure 6.12. General workflow of computerized craniofacial reconstruction techniques[184].

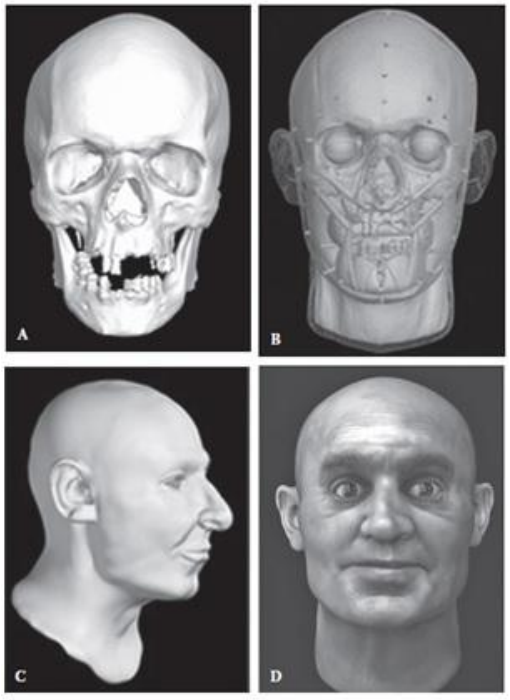


Figure 6.13. Computerized three-dimensional facial reconstruction. A digital model of the skull is produced from CT or laser scan data (A) and the facial musculature attached using three-dimensional modeling software (B). The facial reconstruction can be visualized as a "virtual" clay head (C) or skin, hair, and eye textures and colors can be added to create a more realistic three-dimensional model (D)[212].

7 Fingerprints

Fingerprint identification, also known as Dactyloscopy, is a process in which two samples of friction ridge skin impressions are compared in order to determine if they could have come from the same individual. Fingerprints have been used for personal identification since the 19th century[236]. Their use is based on the assumptions that they are unique to each individual and do not change throughout life[237]. Galton calculated that the probability of finding identical prints was 1 in 64 billions[238].

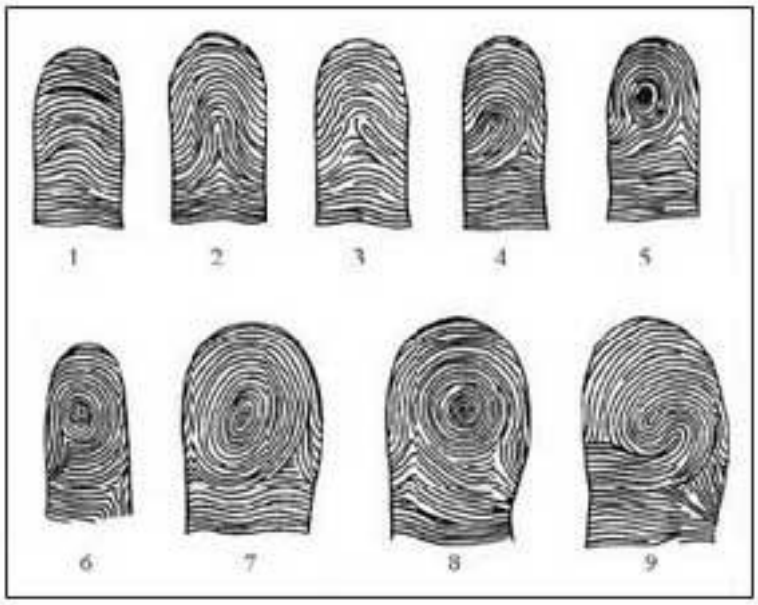


Figure 7.1. .Purkinje's fingerprint patterns[239].

In 1823, John Evangelist Purkinje described nine fingerprint patterns in his thesis[239] (Figure 7.1). The first to realize the individuality of fingerprints was Sir William Herschel, who, in 1858, started using handprints on contracts[240]

(Figure 7.2). Herschel never published his ideas, so it wasn't until 1880 when Faulds published a letter in *Nature* that the importance of fingerprints in forensic science as means of personal identification was first discussed[236]. Faulds discussed a method of classification and suggested the use of printers ink as means of obtaining fingerprints. In 1888, Sir Francis Galton, who had a great interest in fingerprints as means of personal identification, presented his research "Personal Identification and Description"[241]. In his presentation Galton produced a print of the right forefinger and right middle finger of Herschel taken in 1860 and of the same fingers taken in 1888, demonstrating the persistence of ridge characteristics. Galton continued his study of fingerprints trying to prove that they were a reliable method for identifying individuals and in 1892 he published his first book, *Fingerprints*, discussing fingerprint patterns, methods for recording them, and a classification into three groups: arches, loops, and whorls[238]. Intrigued by Galton's work, Sir Edward Henry developed a new classification system. Henry's system consisted in an elaborated method of indexing fingerprints, facilitating the identification process[242]. In the early 20th century, law enforcement agencies considered fingerprints a valid method for personal identification and criminal fingerprint databases were created[243].

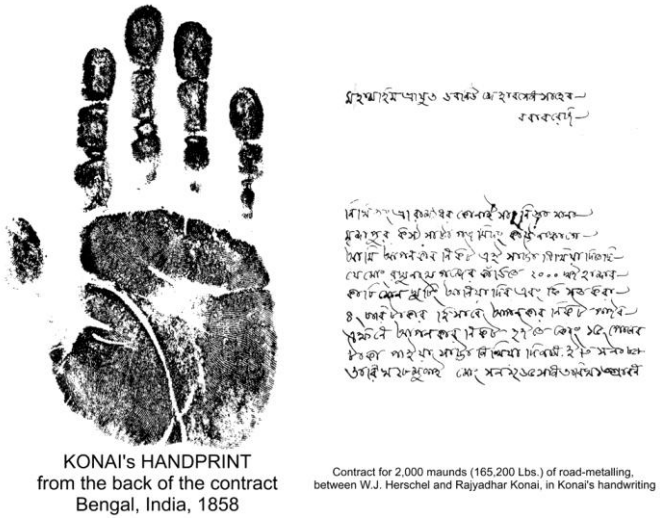


Figure 7.2. Herschel's first contract where a handprint was used as a signature[240].

7.1 Fingerprint Patterns and Characteristics

There are three main fingerprint patterns: loops, whorls and arches[237] (Figure 7.3). The FBI suggests a sub-division of these groups, with a total of 8 patterns[244] (Table 7-1).

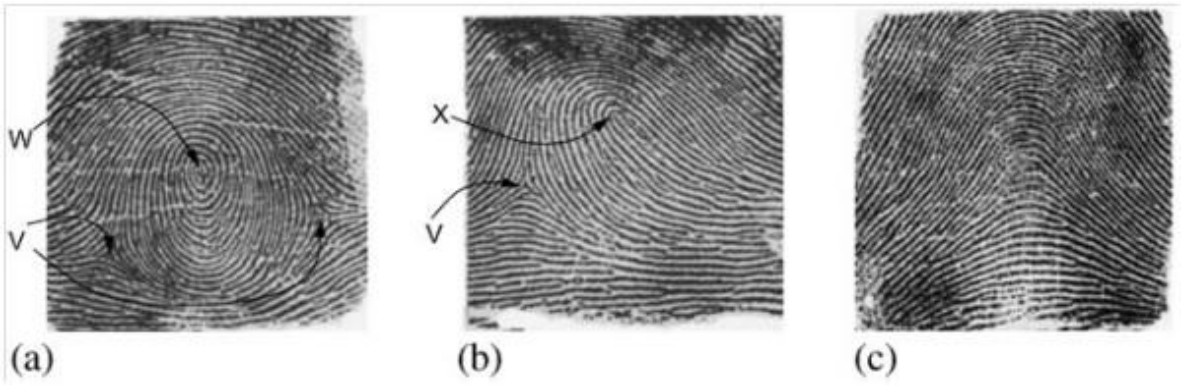


Figure 7.3. The most frequently occurring fingertip patterns: (a) whorl, (b) loop and (c) arch. A whorl is characterized by a target/spiral (W) and two triradii (V,V), loops by a Roman arch structure (X) and one triradius (V)[237].

I. ARCH	II. LOOP	III. WHORL
a. Plain arch.	a. Radial loop.	a. Plain whorl.
b. Tented arch.	b. Ulnar loop.	b. Central pocket loop.
		c. Double loop.
		d. Accidental whorl.

Table 7-1. FBI fingerprint patterns[244].

Fingerprints patterns are formed by the friction ridges on the fingertips and by the depressions between those ridges. Friction ridges develop between the 9th and the 24th week of gestation[237]. It has been shown that there is a link between volar pads and the patterns of friction ridges[245]. Volar pads are temporary mesenchymal eminences that form around the 7th week of gestation and start to regress during the 10th week. During this regression the epidermis folds into the dermis forming the primary ridges[246].

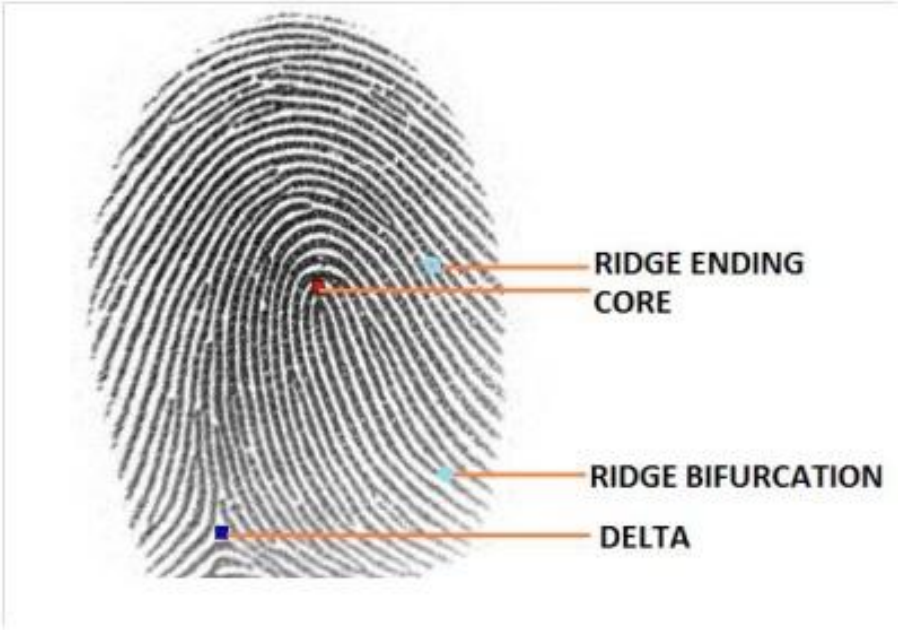


Figure 7.4. Global and local ridge features[247].

The patterns formed by the ridges contain global features, called singular points, and local features, called minutiae[248, 249] (Figure 7.4). These features are essential in the comparison and identification process. Singular points include delta, a point on a ridge at or in front of and nearest the center of the divergence of the type lines, and core, the center of the finger impression[244]. Henry was the first to introduce the concept of singular points as an aid in fingerprint classification. He found that whorl fingerprints have two delta points and one or two core points[242]. Minutiae, also known as Galton's details, include ridge endings and bifurcations[238]. There are a few hypothesizes regarding the process of minutiae formation. One hypothesis suggests that the finger rapidly expands during primary ridge growth causing the separation of the existing ridges. New ridges form to fill the gaps created by the separation

of the old ridges, creating bifurcations[250]. Another hypothesis suggests a chemical reaction caused by hormones circulating through capillaries before ridge formation[251]. The most recent hypothesis links friction ridge morphogenesis to mechanical instability[237].

Ridge density is another characteristic that can help in the identification process. Studies have shown that women have a higher ridge density and finer epidermal ridge detail than man. Acree studied 400 random fingerprints: 100 Caucasian males, 100 Caucasian females, 100 African American males and 100 African American females, all between the ages 18-67. The results of this study show that women have a significantly higher ridge density. A ridge density of 11 ridges/25 mm² or less is more likely to be of male origin while a ridge density of 12 ridges/25 mm² or more is most likely to be of female origin[252]. Two studies conducted by Nayak et al. in the Indian population and in the Chinese and Malaysian populations showed similar results to those obtained by Acree[253, 254]. Nayak's results showed a slightly higher ridge density value for males and females, suggesting not only sex differences in ridge density but also racial differences.

7.2 Fingerprint Recovery Techniques

There are three types of fingerprint impressions: latent, known and plastic. Latent fingerprints are two dimensional reproductions of the friction ridges of the finger on an object created by secretion of the eccrine glands or by contaminants, such as oil or blood, transferred from the friction ridges onto a surface. These impressions can be made visible using alternate light sources, chemical techniques, or fingerprint powders. Known fingerprints are the

intentional reproduction of the friction ridges of the fingers onto a fingerprint card or an appropriate contrasting surface. They can be recovered using black printer's ink, chemical methods, or by scanning the finger and creating a digital fingerprint image. Plastic fingerprints are impressions made using materials, such as wax or putty, which retains the shape of the ridge details[255].

Recovering fingerprints from the living can be achieved by applying black ink to the finger's skin surface and pressing the inked skin on a fingerprint card[256]. Black ink can also be used for recovering fingerprints from recently deceased subjects. The ink can be applied to the deceased's fingers using an ink roller[257].

Recovering fingerprints from a decomposed body could be difficult as the friction skin is very fragile. If it is not possible to apply ink because the friction skin is rubbery and separating from the underlying tissue, the skin should be removed, cleaned and dried. If the skin is too soft or fragile it should be placed in formaldehyde to harden it. The friction skin of each finger is then placed on the technician's gloved finger in order to ink and record it[244]. In cases of advanced decomposition, when the outer layers of the skin have been destroyed, friction ridge details may be visible only on the underside of the friction skin and on the dermis. In these cases the skin is inverted and inked[244].

In cases of maceration, when the fingers are immersed in water for a long period of time, the skin on the fingers absorbs water, swells, and after a few hours loosens from the flesh. If the macerated skin is still intact, it should be cleaned, wiped with alcohol, dried and then inked. If the skin is broken and

loose, it should be removed, cleaned, placed in alcohol, stretched over the technician's finger to smooth the pattern and inked. When the skin is intact but very wrinkled and hard, tissue builder should be injected to round out the pattern area[244].

When the skin is desiccated, rehydration with a solution of sodium or potassium hydroxide is used in order to distend and soften the skin. This solution should be used with caution as it can result in the destruction of the flesh[244]. A less destructive method could be used. This method uses silicone casts (Mikrosil) to record the friction ridge detail in cases where the skin is desiccated[258].

When the body is severely burned, an examination of the fingers should be performed before removing the body in order to determine if the removal of the body could damage the fingers. If the skin is wrinkled but still flexible, tissue builder is injected into the bulbs. If the skin cannot be removed easily, the pattern area is cut off, soaked in xylene to soften, and then placed on the technician's finger and inked. When the fingers are extremely charred, photographing the finger using side lighting is the only method of recording fingerprints[244].

7.3 Comparison and Identification

The identification process requires the comparison of postmortem fingerprints to antemortem fingerprints. The method used for identification is based on analysis, comparison, evaluation, and verification (ACE-V)[259-262].

Analysis consists in examining the print and breaking down the information into three levels of detail[261]. The first level includes the overall ridge flow and pattern type. The second level includes ridge path. Level three includes ridge shapes and the structure and location of the pores[263].

Side by side comparison of the friction ridge impressions is the next step. All three levels of detail should be compared by the examiner[259, 261, 263].

In the evaluation step, the examiner needs to reach a conclusion based on the results of the analysis and comparison. The Scientific Working Group on Friction Ridge Analysis Study and Technology (SWGFAST) defined three possible conclusions that can be reached by the examiner[259]:

- The conclusion of individualization can be made when the examiner determines that the information present in the two impressions matches.
- Exclusion can be reached when the information present in the two impressions does not match.
- An inconclusive decision is made when it is not possible to reach a conclusive comparison because of poor quality or the lack of a comparable area in the exemplar.

The last step, verification, is not technically part of the identification process. It is a form of peer review, ensuring the accuracy of the results. All individualizations need to be verified, but verification is optional for exclusion or inconclusive decisions[259].

7.4 Automated Fingerprint Identification System (AFIS)

AFIS is a storage, search, and retrieval system for fingerprints and palm prints. AFIS systems use algorithms that are based on the friction ridge details, allowing the comparison of a fingerprint with millions of prints that are in the database in seconds. Although these systems are accurate, human verification is necessary for all AFIS match results[264].

Many states have their own AFIS. In Europe, the European police agencies are required by a European council act[265] to open their AFIS's to each other. These systems are used primarily in the identification of criminals, but are also used in the identification of the dead.

AFIS has an important role in human identification in mass disasters due to the possibility to compare a large number of prints in a short period of time. In 2004, a tsunami hit the coast of Thailand, killing over five thousand people. Antemortem records for those presumed dead were requested from worldwide agencies, entered into AFIS, and compared to postmortem prints recovered from the victims. Numerous identifications were made using the automated system.

8 DNA Profiling

DNA profiling is a technique used in forensic science to identify individuals by their DNA characteristics. This technique was developed in 1985 by Alec Jefferys who discovered that particular regions of DNA, called polymorphic regions, contained repeated DNA sequences and were highly variable between individuals[266-268]. Jeffreys developed a method that uses restriction fragments length polymorphism (RFLP) to study these repeated sequences and perform human identity tests[268].

The DNA profiling technique developed by Jeffreys was first used in paternity and criminal cases and only a few years later it was applied as a method for human identification. In 1992, Jeffreys used this technique to identify skeletal remains exhumed in Brazil in 1985 as those of Dr. Josef Mengele[269].

The process of DNA profiling can be divided into three major steps[270] (Figure 8.1). The first step, biology, consists in the extraction, quantification and PCR amplification of DNA. The second step is technology. This step requires that the PCR products be separated and detected in order to determine the genotype. In the last step, genetics, the genotype (DNA profile) is compared to other DNA samples, taken for example from family members, and a conclusion of inclusion or exclusion is reached.

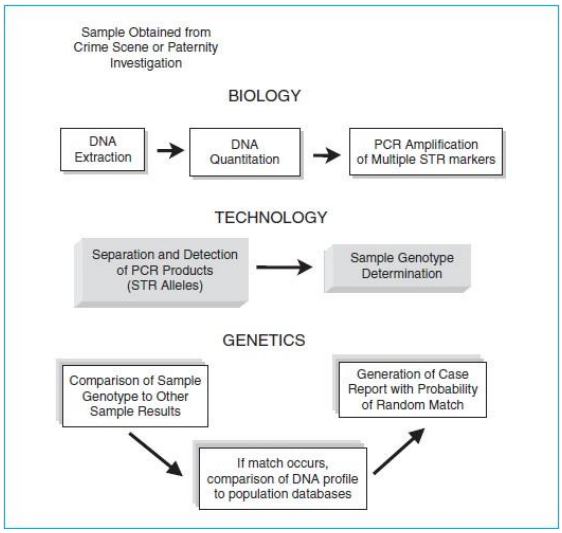


Figure 8.1. Overview of biology, technology, and genetic components of DNA typing using short tandem repeat (STR) markers[270].

8.1 DNA Structure and The Human Genome

The human genome is the entire genetic information of an individual. It is composed of nuclear and mitochondrial DNA. DNA (Deoxyribonucleic acid) is a double strand molecule that contains all of our genetic material. The two strands of DNA are composed of nucleotides. Each nucleotide is composed of a monosaccharide sugar (deoxyribose), a phosphate group, and a nitrogen base that could be either guanine (G), thymine (T), adenosine (A), or cytosine (C) (Figure 8.2). Hydrogen bonds bind the nitrogen bases of the two strands, creating a double-stranded molecule. Watson and Crick found that hydrogen bonds can bind only A with T and G with C[271] (Figure 8.3).

Nuclear DNA (nuDNA) contains approximately 3.2 billion base pairs (bp)[272, 273] organized in 22 pairs of autosomal chromosomes and one pair of sex

chromosomes and located in the cell nuclei. It is inherited from both parents and only two copies of nuDNA are found in each cell, one from each parent. Mitochondrial DNA (mtDNA) is a small circular molecule of DNA, containing only 16,569 bp, located in each mitochondrion[274]. It is inherited only from the mother and is found in multiple copies in each cell.

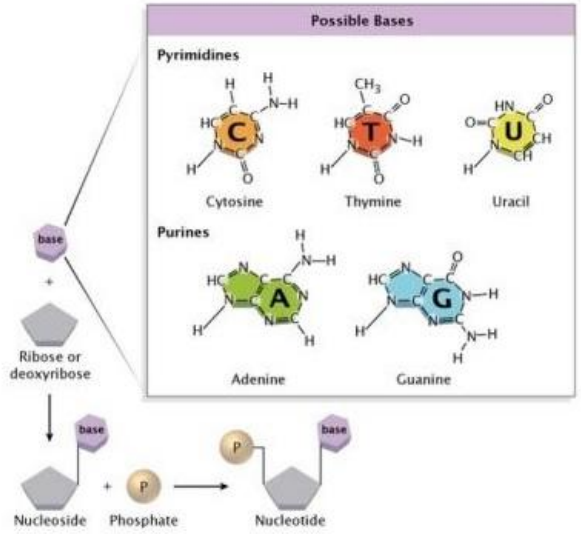


Figure 8.2. The chemical structure of a nucleotide[275].

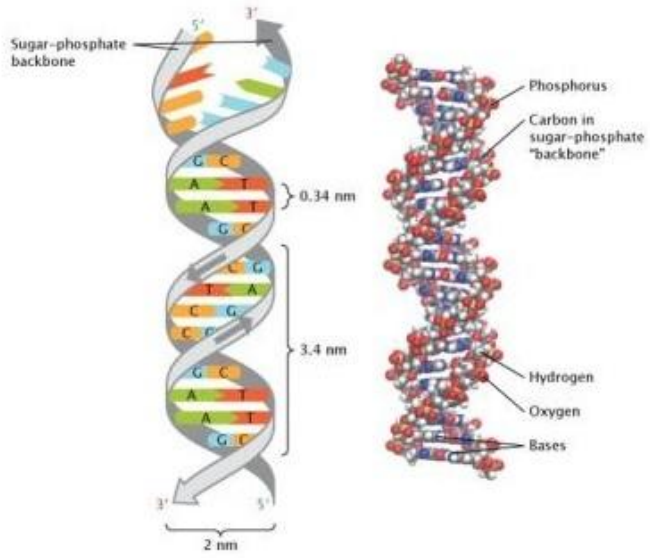


Figure 8.3. The double-helical structure of DNA[275].

8.2 DNA Polymorphisms

More than 99.5% of DNA sequences are identical between individuals[273]. Polymorphism is defined as any sequence variant present at a frequency of 1% or higher in a population[276]. There are two types of DNA polymorphisms: length and sequence polymorphisms. These polymorphisms occur more frequently in non coding regions, which represent ~98% of the genome, and do not have an effect on the phenotype. Polymorphisms that occur in coding regions, however, can either be silent or have an effect on the phenotype. The ABO blood typing system is an example of a polymorphic gene used frequently in forensic science[277].

The polymorphisms discussed in the next paragraphs are also called autosomal markers because they are inherited from both parents. For each

DNA sequence at a specific position on a chromosome (locus), every individual has two variants (alleles): one allele inherited from the mother and one from the father[270, 277].

Length polymorphisms include minisatellites or variable number tandem repeats (VNTRs), and microsatellites or short tandem repeats (STRs). These are DNA sequences that comprise of 2-250 bp of a certain sequence that is repeated numerous times (tandem repeats)[278]. The number of times a certain sequence repeats itself is highly different between individuals (Figure 8.4).

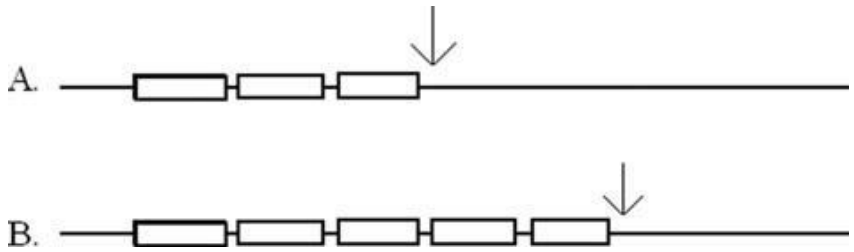


Figure 8.4. Length polymorphisms. Strand A has three units of the repeat sequence whereas strand B has 5. The arrows indicate the recognition site of a restriction endonuclease. Evaluation of the resulting fragments by electrophoresis will show a different DNA migration pattern for strand A compared with strand B[279].

VNTRs are DNA sequences of 8-100 bp, GC rich, which repeat 5-1000 times[280]. They were the first class of polymorphisms used in DNA profiling, as well as in the mapping of the human genome[266, 281, 282]. Restriction fragment length polymorphism (RFLP), where restriction enzymes digest regions at both ends of the repeat sequences, is used to analyze VNTRs. The variable DNA fragments are then separated using gel electrophoresis and detected

using a VNTR probe[283]. The large amount of good quality DNA needed for the analysis of VNTRs has limited their use in DNA profiling[277].

Due to the limitations of VNTRs, their use in DNA profiling was replaced with STRs analysis, which is the most common method used today. STRs are short DNA sequences of 3-7 bp repeating 5-15 times[284]. STRs are much shorter than VNTRs. This characteristic allows the analysis of degraded DNA by amplifying very small amounts of DNA using the polymerase chain reaction (PCR) technique[285, 286]. The STRs most used for personal identification have tetranucleotide repeats[287]. A large number of STRs have already been identified[284, 288, 289]. The FBI's DNA database, Combined DNA Index System (CODIS), consists of 13 core STR loci that are used for personal identification (Figure 8.5). Interpol uses 10 STR loci in the UK and Europe[283, 290-292].

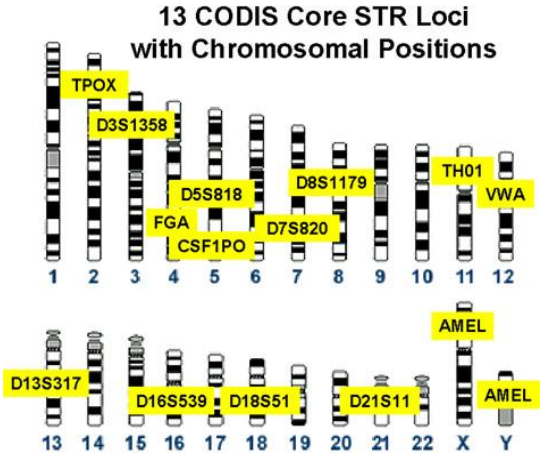


Figure 8.5. CODIS STR loci[293].

The second group of DNA polymorphisms is sequence polymorphisms, or single nucleotide polymorphisms (SNPs). SNPs are the result of a change, deletion or insertion of a single nucleotide and they represent the most abundant group of polymorphisms in humans[277, 294-296] (Figure 8.6). More than 1.4 million SNPs have already been identified[297].

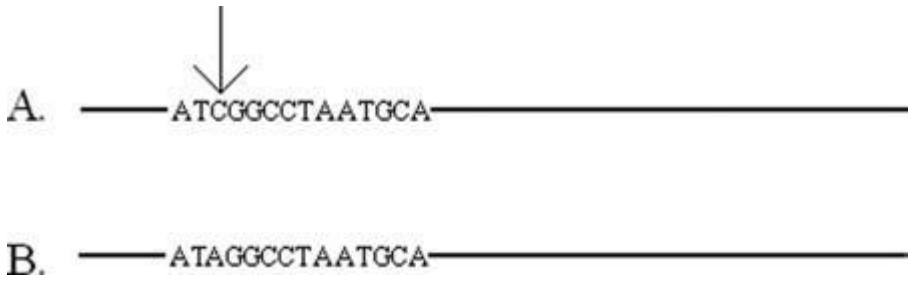
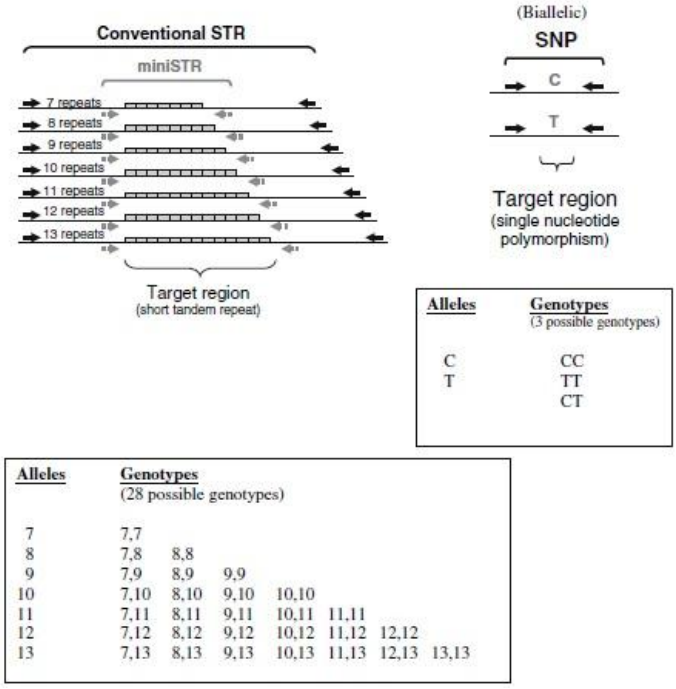


Figure 8.6. Sequence polymorphisms. Strand A depicts a short sequence of DNA that is recognized by a restriction endonuclease at the arrow. Strand B has a different base at its third position that results in no recognition by a restriction endonuclease. Evaluation of the two strands by electrophoresis shows different migration patterns[279].

The use of SNPs in forensic science is limited to cases where the DNA samples available for analysis are highly degraded and their length is smaller than the required length for STR typing. In comparison to STRs, a smaller DNA sample is needed when using SNPs because the target region of the SNP markers is much smaller (Figure 8.7). SNPs have a very low mutation rate. They change once every 10^8 generations, making them very useful in estimating the ethnic origin of a sample[277, 296, 298, 299]. One of the most important disadvantages of using SNPs instead of STRs is their low discrimination power. 40-60 SNPs are needed in order to reach the same levels of discrimination of the 13 STR loci used today because while STRs are very polymorphic and have numerous alleles, SNPs have only two variants in

each locus[277, 295, 299-301] (Figure 8.7). The low number of possible alleles decreases the possibility of distinguishing multiple contributors in a mixture of samples[298, 299].



Alleles	Genotypes (3 possible genotypes)
C	CC
T	TT
	CT

Alleles	Genotypes (28 possible genotypes)						
7	7,7						
8	7,8	8,8					
9	7,9	8,9	9,9				
10	7,10	8,10	9,10	10,10			
11	7,11	8,11	9,11	10,11	11,11		
12	7,12	8,12	9,12	10,12	11,12	12,12	
13	7,13	8,13	9,13	10,13	11,13	12,13	13,13

Figure 8.7. Comparison of (A) STRs and (B) SNPs in terms of the number of possible allele combinations and relative size of the target region[298].

Table 8-1 summarizes the advantages and disadvantages of the various polymorphisms used as biomarkers in forensic science[302].

Biomarker	*Advantages*	*Disadvantages*
VNTR	Mostly polymorphic Ease of analysis of mixed DNA profiles High differentiation resolution power	Less forensic validated loci The laboratory process takes longer (~5–9 days) Low chance of success for degraded DNA Low sensitivity (~5–500 ng)
STR	Rapid and semiautomatic analysis (~1–2 days) Advantageous than VNTR in degraded samples (miniSTR) High sensitivity (~0.1–2 ng DNA) Higher discrimination power than SNPs High multiplex capacity Availability of commercial kits	Less polymorphic than VNTRs Higher mutation rates than SNPs Difficult analysis due to stutters and artifacts Limited to the determination of ethnicity No phenotype information
SNP	High chance of success in degraded DNA samples Low mutation rate, protected for generations High ethnicity information and phenotype data Multiplex capacity is high (>1000 SNP microarray technique) Fast and fully automated possible analysis	Low discrimination power per allele No forensic validated commercial kit Lower sensitivity than STRs Difficulty in analysis of mixed samples

SNP, single-nucleotide polymorphism; STR, short tandem repeat; VNTR, variable number tandem repeat.

Table 8-1. Advantages and disadvantages of genetic biomarkers[302].

8.3 ABO Blood Group System

The ABO blood group system was discovered in 1900 by Landsteiner[303]. Landsteiner found three different blood types: A, B, and O. The fourth type, AB, was found in 1902 by von DeCastrello and Sturli[304]. The ABO gene locus is located on chromosome 9 and has three main allele forms (A, B, O). Seven nucleotide substitutions differentiates the A and B alleles, while a single nucleotide deletion differs the O allele from the A allele[305, 306] (Figure 8.8). The four blood groups are characterized by the presence of specific antigens on the surface of the red blood cells and antibodies in the serum. Blood type A, for example, has antigen A on the red blood cells and anti-B antibodies in the serum. Each group has numerous variants based on the amount of antigens on red blood cells[307, 308] (Table 8-2). Numerous methods are available for ABO blood typing. These include haemagglutination, which uses antibodies

that bind to the blood antigens and cause agglutination of the red blood cells, or DNA sequencing methods such as PCR and PCR-RFLP[309].

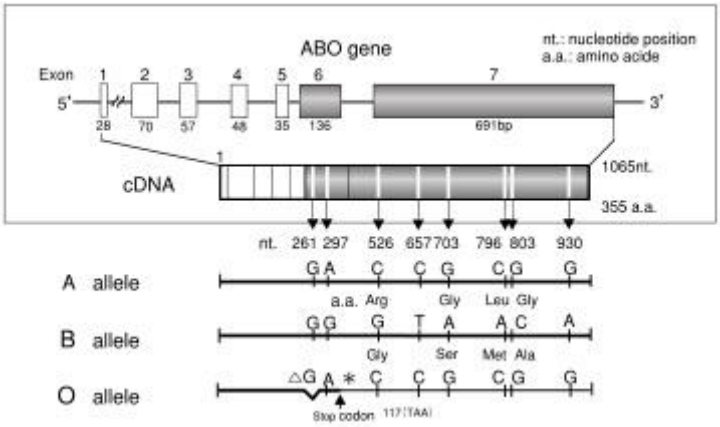


Figure 8.8. Structure of the ABO gene locus and nucleotide sequence of A, B and O alleles. Diagram of exon organization of the protein coding sequences (upper shaded). *; Entirely different deduced amino acid sequence in O alleles due to frame-shifting caused by a single base deletion (lower)[310].

Blood group type		Expression
A_1	adult	810,000 ~ 1,170,000
A_1	newborn	250,000 ~ 370,000
A_2	adult	240,000 ~ 290,000
A_2	newborn	140,000
A_1B	adult	460,000 ~ 850,000
A_1B	newborn	240,000 ~ 290,000
A_2B	adult	120,000
A_3		7,000 ~ 100,000
A_x		1,400 ~ 10,000
A_{end}		1,100 ~ 4,400
A_m		200 ~ 1,900
A_{el}		100 ~ 1,400
B	adult	610,000 ~ 830,000
B	newborn	200,000 ~ 320,000
A_1B	adult	310,000 ~ 560,000

Table 8-2. Expression of ABO antigens per red blood cell surface[310].

Of the four possible blood types, nearly 40% of the population is blood type A and another 40% is type O. The low number of group types and their frequency in the population make this method more effective at excluding an individual or narrowing down the possibilities than positively identifying him. Therefore, in cases of personal identification, DNA typing methods have replaced the ABO blood group system[270, 311].

8.4 mtDNA and the Y Chromosome

Mitochondrial DNA (mtDNA) and the Y chromosome are called lineage markers. These markers, unlike the autosomal markers discussed earlier, are passed only from one parent: mtDNA is passed from the mother and the Y chromosome is passed from the father and is found only in males[270] (Figure 8.9).

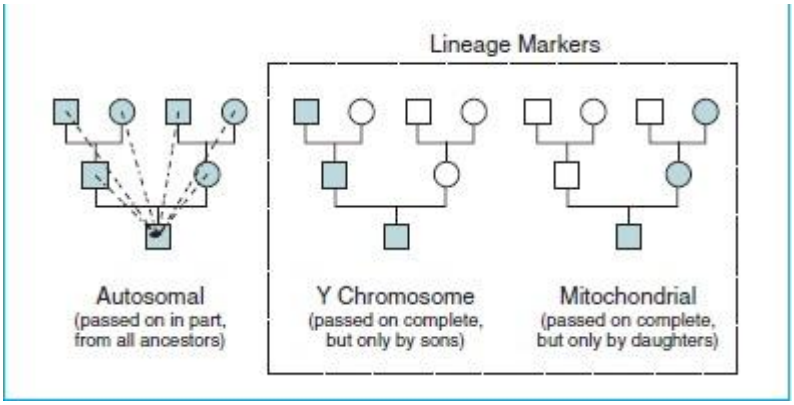


Figure 8.9. Illustration of inheritance patterns from recombining autosomal genetic markers and the lineage markers from the Y chromosome and mitochondrial DNA[270].

MtDNA is a circular molecule containing 16,569 bp with two 350 bp hypervariable regions[270, 312]. Thousands of copies of mtDNA are present in every cell, making it more likely to obtain mtDNA than nuDNA from highly degraded remains[313, 314]. Since mtDNA in inherited only from the mother, only one copy passes to the children, and since the mtDNA does not go through recombination, the sequence is identical in all the maternal relatives. This characteristic could be used to determine maternal family relationship, but since the mtDNA sequence is not unique it is harder to make a certain identification[270, 315].

The Y chromosome is inherited from the father and contains a large number of STRs and SNPs[316-318]. Since the Y chromosome is found only in males it is helpful in determining the sex of an individual. Like mtDNA, the Y chromosome passes from the father to the children without recombination and is almost identical between individuals in the paternal lineage. A match between two Y chromosomes indicates that there is a paternal relationship between the two individuals, but it does not necessarily mean that the two samples came from the same individual. The polymorphisms present in the Y chromosome can help determine if two Y chromosomes came from the same individual or not[318].

8.5 Extraction of DNA From Biological Samples

DNA can be found in any type of biological material[277, 319]. Bone, saliva, muscle, and skin are examples of biological remains from which DNA can be

extracted and used for DNA profiling (Table 8-3). The type of material available and the quality of the DNA sample depend on the time elapsed since death and the state of degradation[277]. In some cases bones or teeth can be the only source of DNA. Reference samples should also be taken from family members in order to compare them with the DNA collected from the biological samples.

After collecting the biological sample, DNA needs to be extracted and separated from the other cellular components. Some cellular proteins, for example, protect the DNA inside the cell and inhibit the ability to analyze it[270]. The most common DNA extraction methods currently used include Chelex extraction[320], organic or phenol extraction[270, 319], silica based extraction[270, 321], and FTA paper[270] (Figure 8.10).

Crime Scene Samples	Human Remains
Blood	Teeth
Semen	Bones
Hair	Muscle
Feces	Skin
Epithelial cells — shed skin cells:	Hair
Saliva	
Dandruff	Reference Samples
Clothing	Buccal swabs/venous blood from:
Cigarette butts	Parents
Touch DNA	Children
	Siblings
Suspect Samples	Maternal relations
Buccal swabs	Paternal relations
Pulled hairs (containing roots)	
Venous blood	Artifacts:
	Hair brushes
	Toothbrushes
	Razors, etc.

Table 8-3. Types of biological material that can be used for DNA profiling[277].

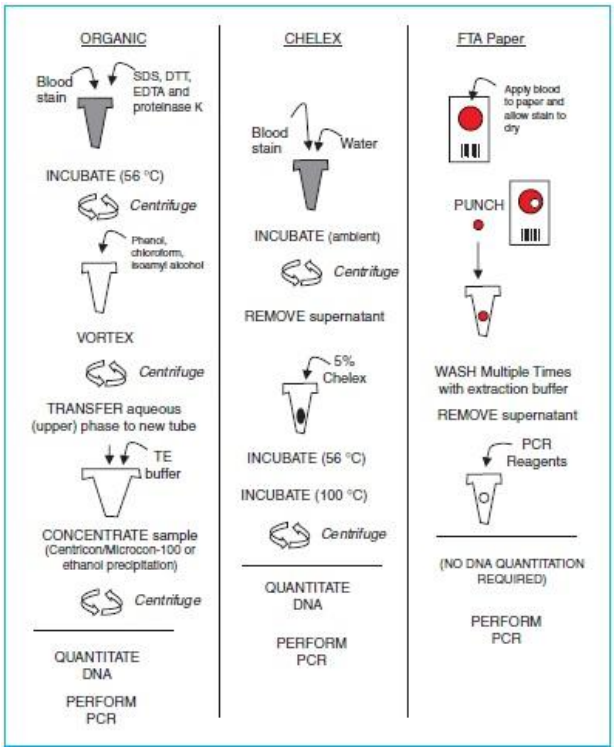


Figure 8.10. Schematic of commonly used DNA extraction processes[270].

The Chelex method uses an ion exchange resin that is added to the sample and then boiled in water for a few minutes causing the rupture of the cells. Chelex is composed of paired iminodiacetate ions that bind polyvalent ions like magnesium, removing them from the reaction. The removal of magnesium from the reaction prevents the degradation of DNA by inactivating the DNAse enzymes. This method is considered to be more rapid than others, less expensive, and with less risk of contamination. It is used to extract DNA from samples such as bloodstains, hair, tissues, and bone[270, 322].

FTA paper is used for extracting DNA from blood and saliva samples[323, 324]. It is a cellulose based paper containing chemicals that lyse the cells and

prevent DNA degradation and bacterial growth[325]. Using FTA paper, DNA can be preserved for a long period of time in room temperature. One of the advantages of this method is that quantification is not necessary in order to reach consistent results[270]. This method, however, is unable to remove PCR inhibitors from degraded tissue samples[321].

The organic extraction method uses sodium dodecylsulfate (SDS) and proteinase K to break the cells and the proteins that protect the DNA. A phenol and chloroform mixture is then added to separate the sample in an organic phase, containing the proteins, and an aqueous phase which contains the DNA. This method is used to recover DNA from samples containing a small amount of biological material, such as bone and hair[270, 322].

In silica based extraction methods, DNA is absorbed on a silica support with a high concentration of chaotropic salts and the proteins are washed away[270]. The silica surface can not bind DNA fragments that are too short, making these methods impossible to use when the samples are highly degraded[270, 321].

Numerous automated extraction systems have been designed recently. These systems allow the extraction of both small and large quantities of DNA and they are based on a magnetic separation technology[326-329]. Magnetic beads are used to bind the DNA fragments while all the unbound material is washed away.

8.6 DNA Quantification

Determining the quantity of DNA extracted is important for a number of reasons. First, PCR amplification works with a narrow range of DNA. The PCR reaction can fail to amplify the DNA if too little is used or it can result in split peaks if too much is used[270]. Furthermore, when using a destructive analytical method it is important to determine the total amount of DNA available and try to preserve some of it for retesting[330]. Most STR kits, for example, require ~1ng of DNA which correspond to ~333 copies of each locus that will be amplified[270].

Various quantification methods have been developed over the years[330, 331]. The slot blot method is one of the most common methods used today[332, 333]. DNA is captured on a nylon membrane and then a human specific probe is added. The intensity of the signal is visually or digitally compared with calibration standards (Figure 8.11). Real-time PCR is another method used to quantify DNA. This method measures the quantity of DNA while it is being amplified[270, 334, 335]. The presence of inhibitors extracted with the DNA, highly degraded DNA, and small quantities of DNA can cause the PCR reaction to fail. Methods using fluorescent dye the binds to the DNA strand, like PicoGreen, are also available[336].

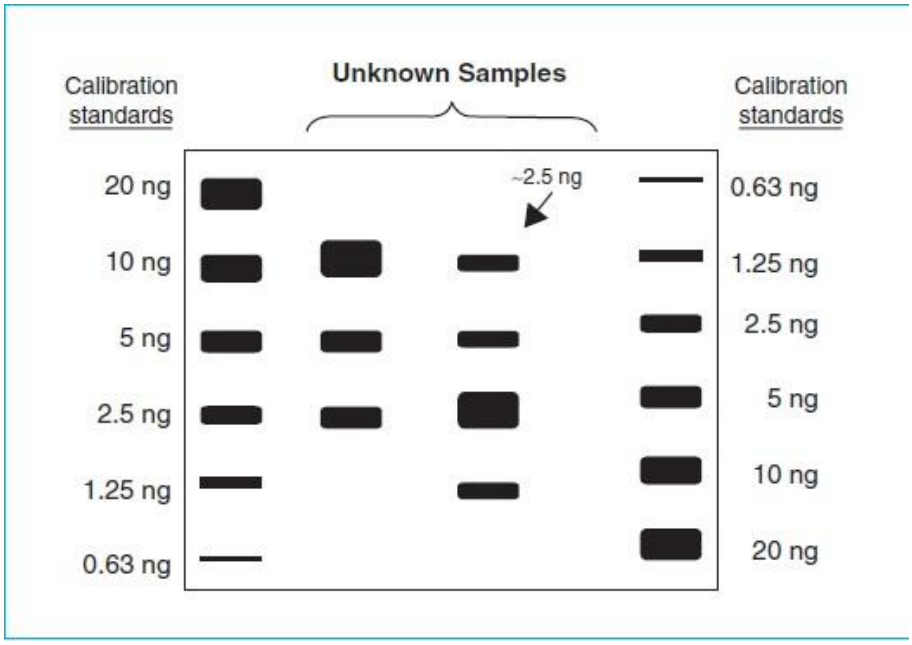


Figure 8.11. Illustration of a human DNA quantification result with the slot blot procedure. A serial dilution of a human DNA standard is run on either side of the slot blot membrane for comparison purposes. The quantity of each of the unknown samples is estimated by visual comparison to the calibration standards. For example, the sample indicated by the arrow is closest in appearance to the 2.5 ng standard[270].

8.7 DNA Amplification

DNA amplification using the polymerase chain reaction (PCR) was developed in 1985[337]. PCR is a technique that can amplify a specific DNA sequence millions of times, making it possible to analyze small amounts of degraded DNA[337, 338]. It requires a DNA template, primers, nucleotides, and DNA polymerase. Each PCR cycle consists of three phases (Figure 8.12). The reaction is first heated to 95°C to separate the two DNA strands, a process called denaturation. The temperature is then lowered to 50-60°C, which allows the primers to bind to the target DNA sequence, a process called annealing.

After the primers bind to the DNA, the temperature is increased to 72°C. This temperature allows the DNA polymerase to extend the primers by adding nucleotides to the new DNA strand. The cycle is normally repeated between 28 and 32 times. Normally, prior to PCR amplification, the PCR primers are labeled with fluorescent dyes. During the amplification process the dye is incorporated in the PCR products, facilitating their detection[277, 339].

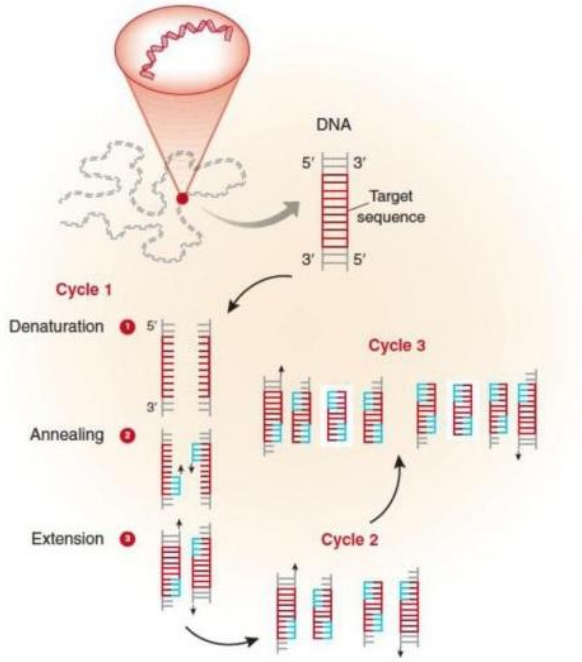


Figure 8.12. Schematic presentation of the PCR principle[339].

Multiplex PCR and real-time PCR are two methods that use the classical PCR reaction. Multiplex PCR allows the simultaneous amplification of two or more DNA regions by adding multiple primers[340] (Figure 8.13). Real-time PCR, as mentioned before, quantifies a targeted DNA molecule while amplifying it. This method uses either a fluorogenic 5' nuclease assay

(TaqMan) or an intercalating dye, like SYBR green, specific for double stranded DNA molecules. TaqMan probes anneal to a specific DNA region. While the DNA strand is being amplified, the Taq polymerase degrades the probe, releasing the fluorescent dye. SYBR green binds all PCR products and therefore it is less sensitive than the TaqMan probe[270].

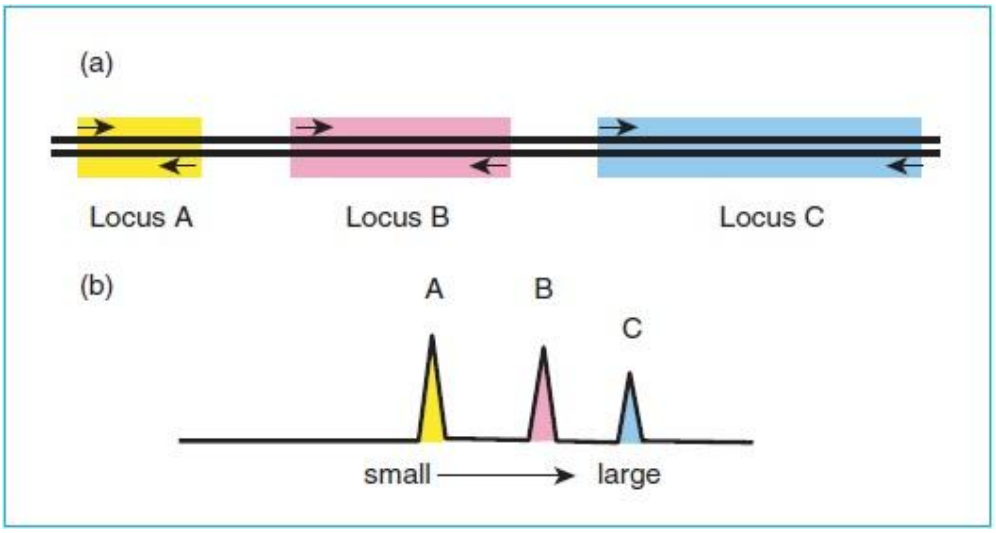


Figure 8.13. Schematic of multiplex PCR. A multiplex PCR makes use of two or more primer sets within the same reaction mix. Three sets of primers, represented by arrows, are shown here to amplify three different loci on a DNA template (a). The primers were designed so that the PCR products for locus A, locus B, and locus C would be different sizes and therefore resolvable with a size-based separation system (b)[270].

Numerous kits for PCR amplification of STR markers, which are the most common genetic markers used in DNA profiling, have been developed[285, 290, 341-343] (Table 8-4). These kits allow the simultaneous amplification of numerous STR markers (multiplexing), including the 13 core STR loci used by the FBI.

Name	Source	Release Date	STR Loci Included
TH01, TPOX, CSF1PO monoplexes (silver stain)	Promega	Feb 1993	TH01, TPOX, CSF1PO
AmpF/STR® Blue	Applied Biosystems	Oct 1996	D3S1358, VWA, FGA
AmpF/STR® Green I	Applied Biosystems	Jan 1997	Amelogenin, TH01, TPOX, CSF1PO
CTTv	Promega	Jan 1997	CSF1PO, TPOX, TH01, VWA (vWF)
FFFL	Promega	Jan 1997	F13A1, FES/FPS, F13B, LPL
GammaSTR	Promega	Jan 1997	D16S539, D13S317, D7S820, D5S818
PowerPlex™ (version 1.1 and 1.2 later)	Promega	Jan 1997 Sept 1998	CSF1PO, TPOX, TH01, VWA, D16S539, D13S317, D7S820, D5S818
AmpF/STR® Profiler™	Applied Biosystems	May 1997	D3S1358, VWA, FGA, Amelogenin, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820
AmpF/STR® Profiler Plus™	Applied Biosystems	Dec 1997	D3S1358, VWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820
AmpF/STR® COfiler™	Applied Biosystems	May 1998	D3S1358, D16S539, Amelogenin, TH01, TPOX, CSF1PO, D7S820
AmpF/STR® SGM Plus™	Applied Biosystems	Feb 1999	D3S1358, VWA, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, TH01, FGA
PowerPlex® 2.1 (for Hitachi FMBIO users)	Promega	June 1999	D3S1358, TH01, D21S11, D18S51, VWA, D8S1179, TPOX, FGA, Penta E
PowerPlex® 16	Promega	May 2000	CSF1PO, FGA, TPOX, TH01, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, Penta D, Penta E, amelogenin
PowerPlex® 16 BIO (for Hitachi FMBIO users)	Promega	May 2001	CSF1PO, FGA, TPOX, TH01, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, Penta D, Penta E, amelogenin
AmpF/STR® Identifiler™	Applied Biosystems	July 2001	CSF1PO, FGA, TPOX, TH01, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D2S1338, D19S433, amelogenin
AmpF/STR® Profiler Plus™ ID (extra unlabeled D8-R primer)	Applied Biosystems	Sept 2001	D3S1358, VWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820
PowerPlex® ES	Promega	Mar 2002	FGA, TH01, VWA, D3S1358, D8S1179, D18S51, D21S11, SE33, amelogenin
AmpF/STR® SEfiler™	Applied Biosystems	Sept 2002	FGA, TH01, VWA, D3S1358, D8S1179, D16S539, D18S51, D21S11, D2S1338, D19S433, SE33, amelogenin

Table 8-4. Commercially available STR multiplexes (fluorescently-labeled)[270].

8.8 Separation of the PCR Products and Determination of the Genotype

The amplification process produces a mixture of different PCR products that need to be separated and measured. Gel electrophoresis is used to separate the DNA molecules based on their size with an electric current[279, 344, 345]. The DNA molecules, which are negatively charged, are put in a gel and migrate towards a positive pole when an electric current is applied. Large molecules

move slower through the gel than small molecules (Figure 8.14). The molecules are labeled with different fluorescent dyes so they can be detected.

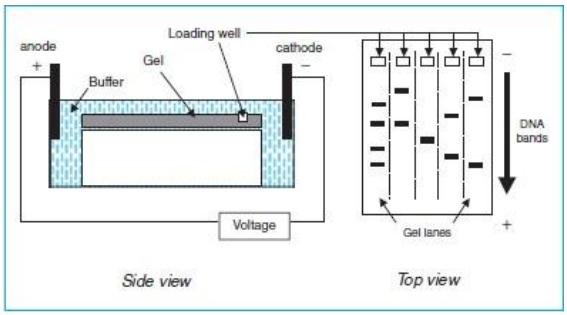


Figure 8.14. Gel electrophoresis[270].

Capillary electrophoresis is the most common electrophoresis method used today[346, 347]. A polymer gel and fluorescent DNA molecules are put in a capillary tube. A laser light shines through the capillary at a fixed position and detects the DNA molecules as they migrate towards the positive pole (Figure 8.15). The data is recorded and each molecule detected is displayed on an electropherogram as a peak. Each peak represents an allele at a specific locus and all the peaks together make a DNA profile[348] (Figure 8.16).

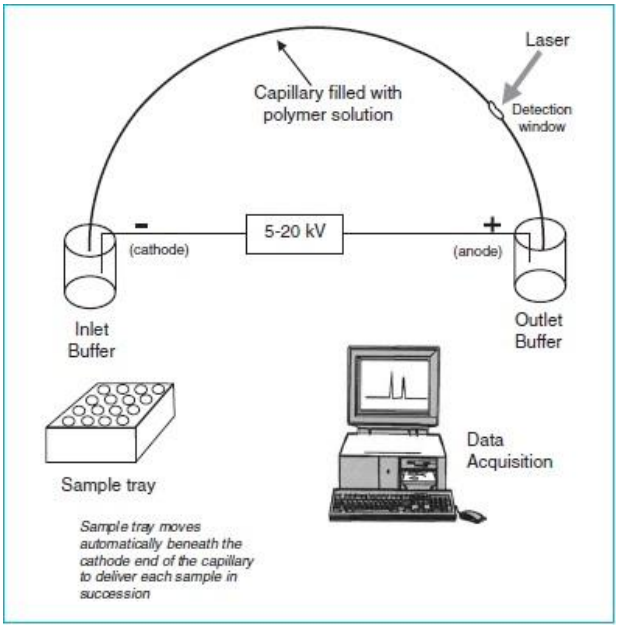


Figure 8.15. Capillary electrophoresis[270].

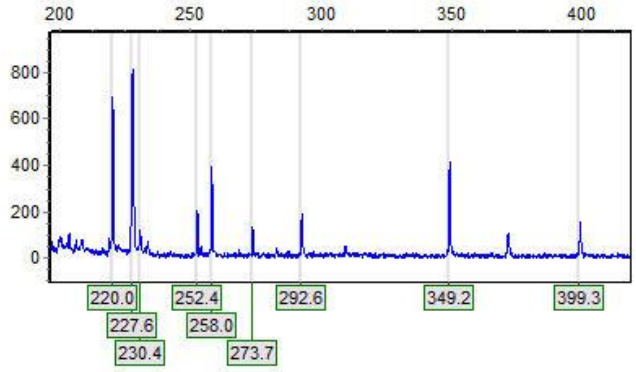


Figure 8.16. DNA electropherogram trace[349].

In order interpret the results and determine the genotype, software programs are used. These programs compare the size of the DNA peaks to a standard allelic ladder in which the size of the alleles in each locus is known, and a

number is assigned to each allele, corresponding to the number of repeats (Figure 8.17).

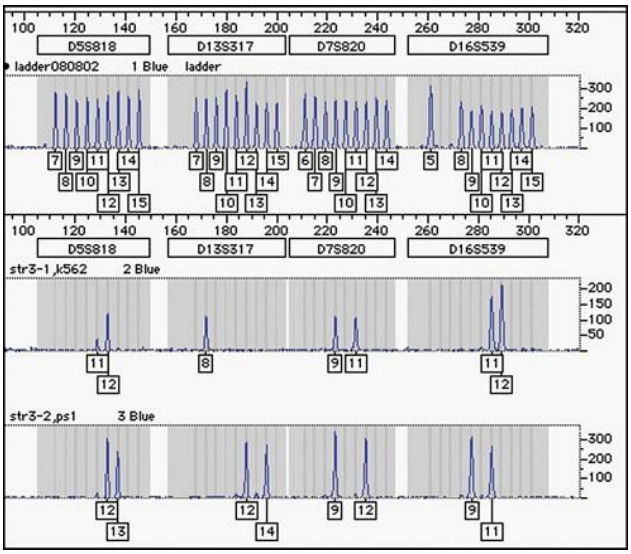


Figure 8.17. Automated DNA identity testing using PCR amplification of short tandem repeats. The top panel is a reference panel showing all possible alleles for a series of four different regions of DNA on different chromosomes. The two lower panels show laboratory-generated data for two different individuals for these four regions[279].

8.9 Comparison and Statistical Analysis of Two DNA Profiles

In order to make a positive or negative identification of an individual using a DNA profile, the profile generated has to be compared to a second profile created from a reference sample. If the two profiles do not match, exclusion is made. However, if the profiles match, a statistical analysis of the match is preformed[350, 351].

The purpose of a statistical analysis in case of a match is to determine the probability that two unrelated individuals have the same profile[351, 352]. The

calculations are done by comparing the sample DNA profile to a population database containing DNA profiles from unrelated individuals of the same ethnic group[353-355]. These databases provide information about the frequency of the common alleles and the genotype frequency for each locus[270] (Table 8-5). Two approaches could be used to calculate the probability match: match probability and likelihood ratio. The match probability presents the probability that the sample DNA profile came from a person unrelated to the individual. This probability equals the frequency of the genotype in the population[351]. The likelihood ratio compares the probabilities of the sample under two different hypotheses:

$$LR = {}^{H_p}\Big/_{H_d}$$

The first hypothesis (H_p) claims that the sample DNA profile came from the individual in question and it always equals 1 (100% probability). The second hypothesis (H_d) claims that the DNA sample is from a different individual than that in question and the probability is equal to the frequency of the genotype in the population. If LR is > 1, then the sample and reference profiles came from the same individual. If however LR < 1, then the two profiles came from different individuals[351].

DNA Profile		Allele Frequency from Database			Genotype Frequency for Locus	
Locus	*Alleles*	*Times Allele Observed*	*Size of Database*	*Frequency*	*Formula*	*Number*
D13S317	11 14	205 29	604	p = 0.34 q = 0.05	2pq	0.03
TH01	6 6	140	604	p = 0.23	p^2	0.05
D18S51	14 16	83 84	604	p = 0.14 q = 0.14	2pq	0.04
				Profile Frequency =		0.000060 1 in 17 000

Table 8-5. Example calculation of the DNA profile frequency or random match probability using alleles from three STR loci[270].

9 Conclusions

The techniques used for human identification require the comparison between antemortem and postmortem data in order to reach a positive identification. Therefore, knowing the possible identity of the decedent is fundamental to the identification process.

The identification of missing persons and human remains from mass disasters is challenging for forensic experts because the possible identity of the decedent is often unknown and so it results impossible to perform any matching with a possible missing person. Furthermore, human remains from mass disasters are often fragmented, commingled, and not well preserved, making it difficult to collect PM data for future comparison.

Osteology and dental profiling methods are widely used to help the forensics team build the biological profile and determine the possible identity of the decedent. These techniques are important in disaster victim identification and identification of unknown bodies of missing persons because they can be used even when the remains are fragmented and not well preserved. Facial reconstruction is possible only in cases where the skull is found in good condition, making it less useful in mass disasters.

Once the possible identity of the decedent has been determined, comparison of dental records, fingerprint analysis, and DNA analysis are the techniques used to reach positive identification. The possibility to collect dental remains, fingerprints, and DNA samples from fragmented and badly preserved remains

makes these techniques very useful in cases of disaster victim identification and identification of missing persons and unidentified bodies.

The numerous techniques available for human identification allow forensic experts to overcome the challenges faced in mass disaster identification and in the identification of missing persons and unidentified bodies and reach positive identification in a large number of cases.

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